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THE INCREASE IN INCIDENCE OF CARCINOMA OF THE LUNG IN DENMARK, 1931 TO 1950.

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In 1947 Clemmesen and Busk reviewed the material of the Danish death certificates with a diagnosis of cancer of the lung from the years 1931 to 1945 (Clemmesen and Busk, 1947). They found an increase in the numbers for males, particularly pronounced in Copenhagen, the capital, less striking in provincial towns, and somewhat smaller for rural areas.

However, the incidence of cancer of the lung among persons subject to routine examination in the Central Tuberculosis Station for the City of Copenhagen did not reflect the heavy increase in mortality found in the city as a whole. An increase in the number of cases aged over 45 did occur, particularly in 1941, but as illustrated in Fig. 1 the values decreased to some extent during the following years. The authors therefore concluded that no increase of the incidence of lung cancer corresponding in gravity to the tripling of the mortality rates for males

TABLE I.—*Cancer of Lung.* (*Revised figures.*) *Central Tuberculosis Station, Copenhagen.*

Year.	Males aged over 45. Cancer.	Males aged over 45. Morbidity rate per 10,000.	Females aged over 45. Cancer.	Females aged over 45. Morbidity rate per 10,000.
1936	6	128·8	0	0·0
1937	2	38·2	0	0·0
1938	6	114·1	1	17·2
1939	9	142·9	1	15·0
1940	8	140·8	0	0·0
1941	18	258·6	1	14·5
1942	21	229·3	2	22·8
1943	26	268·9	3	28·4
1944	20	189·4	1	9·8
1945	22	208·1	1	8·9
1946	43	278·3	3	21·6
1947	34	202·5	7	44·4
1948	44	353·1	4	30·8
1949	37	280·7	3	22·8
1950	54	342·6	4	25·5

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in the capital from 1936 to 1945 was found among the patients of the Central Tuberculosis Station. For this reason it seemed to Clemmesen and Busk (1947) that the apparent increase in lung cancer mortality was to a very large extent conditioned by improvements in diagnostic means. It should be noted that during the years following 1940 lung surgery and systematic bronchoscopy were introduced in Copenhagen, and that the mortality rates for cancer of the lung increased particularly steeply during this period following the increase of cases found in the Tuberculosis Station with a delay of one year.

However, seen in the light of later experience the picture has changed.

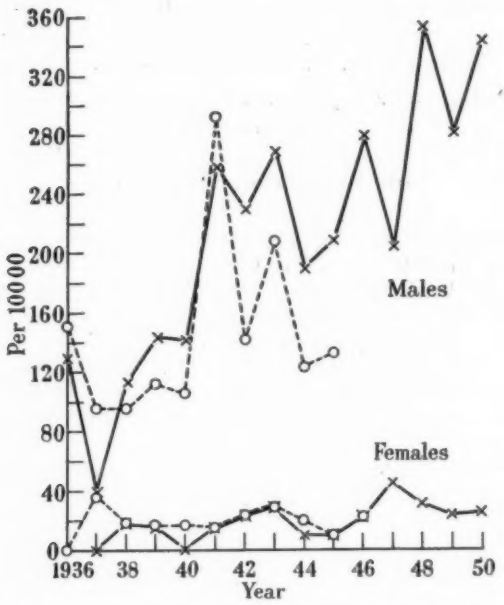

FIG. 1.—Lung cancer in Copenhagen, Central Tuberculosis Station. Incidence per 10,000 examined. Aged over 45.

○-------○ Material of 1947.
×———× Revised material.

Tracing the ultimate fate of all its patients the Central Tuberculosis Station has been able retrospectively to diagnose a number of cases of cancer of the lung, so that the graph illustrating the incidence rates in its material should be changed, as shown in Fig. 1.

During the years up to 1950 the increase in mortality rates for cancer of the lung among men in the various areas of Denmark continued, and from Fig. 2 it appears that the increase following 1940, however steep, was not mainly due to the improvements in diagnostic technique taking place at this time. As we now can see from the slope of the later part of the graph these improvements caused just an acceleration of a continuous increase. Correspondingly, the smaller

irregularity occurring in the graph for women (Fig. 3) at the same time now can be computed to be a statistically significant elevation of this, otherwise horizontal, graph, which is just what would be expected as result of an improvement in diagnostic technique.

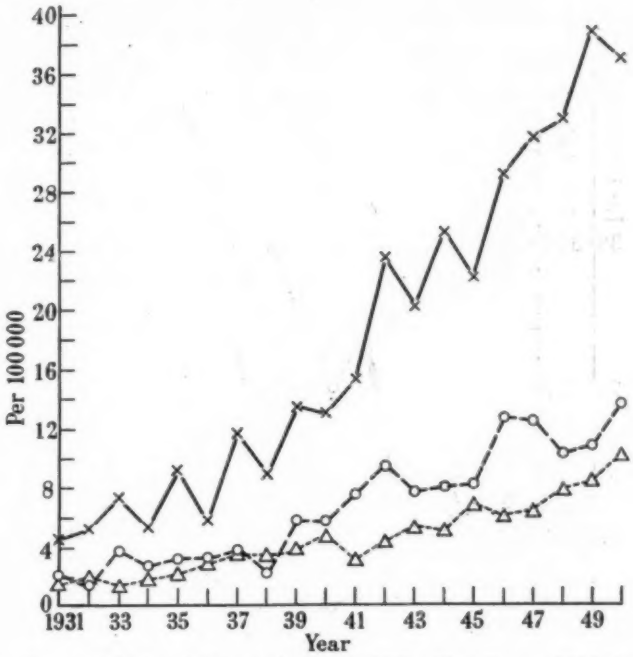

FIG. 2.—Cancer of the lung in Denmark, 1931 to 1950. Mortality per 100,000 males.

×———× Greater Copenhagen.
○— — —○ Provincial towns.
△- - - - - - -△ Rural areas.

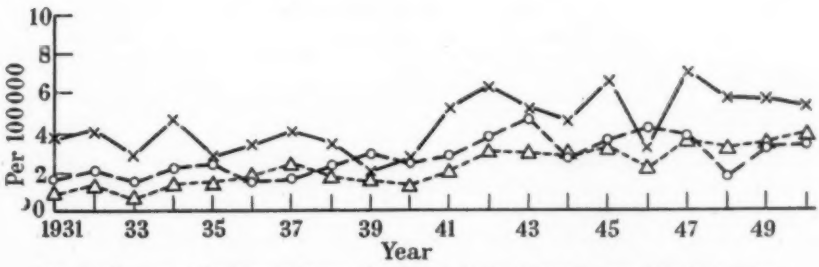

FIG. 3.—Cancer of the lung in Denmark, 1931 to 1950. Mortality per 100,000 females.

×———× Greater Copenhagen.
○— — —○ Provincial towns.
△- - - - - - -△ Rural areas.

Moreover, it appears from Fig. 4 that there is now conformity between the incidence rates for persons examined in the Tuberculosis Station and mortality rates for the city when allowance is made for a difference in time of one year between diagnosis and death, and for an incidence of one hundred times the crude mortality rate among persons aged over 45 and referred to the Tuberculosis Station for chest trouble.

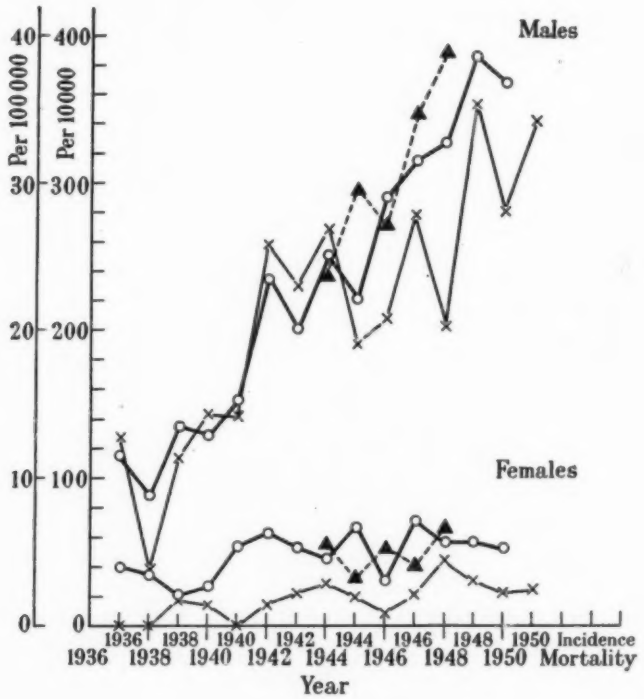

FIG. 4.—Lung cancer.

○———○ Mortality per 100,000 for Copenhagen.
×———× Incidence per 10,000 aged over 45, Central Tuberculosis Station, Copenhagen.
▲— — —▲ Incidence per 100,000, The Danish Cancer Registry, Copenhagen.

Furthermore the material of the Danish Cancer Registry for the years 1943 to 1947 shows that the statistically significant increase in numbers of cases reported from Greater Copenhagen during this period was not followed by any increase in the percentage of cases admitted in hospital, nor of cases histologically examined or subject to post-mortem examination (Clemmesen, Nielsen and Jensen, 1953).

As far as Copenhagen is concerned, it would seem that the three materials provide conclusive evidence that the increase in cases among men diagnosed as cancer of the lung is for the larger part caused by a real increase in the frequency

TABLE II.—*The Danish Cancer Registry* 1943 *to* 1947. *Carcinoma of Bronchus and Lung* (162·1 *and* 163). *Capital.*

Year.	Total number of cases.		Admitted in hospital; percentage of total.		Histologically verified percentage of hospital cases.		Autopsies; percentage of all deaths.	
	Men.	Women.	Men.	Women.	Men.	Women.	Men.	Women.
1943	96	27	94	93	68	72	66	81
1944	129	17	95	88	64	80	62	69
1945	120	23	93	91	65	57	61	65
1946	150	21	91	100	65	81	63	75
1947	172	31	92	81	70	60	60	47
Total	667	119	93	90	67	69	62	66

of the disease, and only to a small extent attributable to improvements of diagnosis, which can be expected to cause a proportional increase for both sexes in the number of cases diagnosed. The possibility that the deciding factor might be an increase in medical attention for all categories of diagnosis can be excluded because the patients from the Tuberculosis Station were unselected and referred directly for diagnosis with a view primarily to a disease different from bronchial carcinoma, and finally were examined in a uniform way.

Further analysis of the material from the Danish Cancer Registry confirm the results from mortality statistics (Clemmesen and Busk, 1947) that the increase in incidence of cancer of the lung among men is particularly strong for the relatively younger age classes (Clemmesen and Nielsen, 1952*a*, 1952*b*). This seems to speak against the assumption that atmospheric pollution is a causative factor of primary importance to cancer of the lung as far as Denmark is concerned. If this were the case we would expect an increase in incidence for all age classes. It might also be expected that the windy climate of Copenhagen could cause regional differences in incidence of the disease within the city analogous to the findings of Stocks (1952) for London, but in fact it appears that the distribution of cancer of the lung among men in Copenhagen varies with the social level expressed in terms of annual house rent, much like cervical cancer of the uterus being most frequent in the classes of subdistricts with the lowest level of house rent, although the single subdistricts show no such parallelism. However, there is no tendency for leeward areas to exceed the weatherside with regard to incidence rates for carcinoma of the lung (Clemmesen and Nielsen, 1951).

Thus, it would seem that the main reason for assuming atmospheric pollution to be causative to cancer of the lung in Denmark would be the excess of cases in towns, particularly in Copenhagen. An explanation of this phenomenon will be given in the following.

Comparisons of age distribution of cancer of the lung between the registration material (Clemmesen and Nielsen, 1952*a*, 1952*b*; Clemmesen *et al.*, 1953) and mortality statistics (Clemmesen and Busk, 1947) will show full conformity, so that the latter material will up to the latest years be conclusive and applicable in an analysis according to the principles used by Korteweg (1927) and applied by him to cancer of the lung (Korteweg, 1951). Figures for the following analysis were published by Clemmesen and Busk (1947), except for those for the later period given in Table III.

TABLE III.—*Deaths from Cancer of the Lung in Denmark, 1946 to 1950.*

Age.	Males.				Females.			
	Capital.	Provincial towns.	Rural areas.	Total.	Capital.	Provincial towns.	Rural areas.	Total.
0–19	—	1	2	3	—	—	—	—
20–24	—	1	2	3	—	—	—	—
25–29	—	—	3	3	—	—	4	4
30–34	2	2	4	8	—	2	1	3
35–39	7	9	3	19	3	1	9	13
40–44	29	16	23	68	2	4	3	9
45–49	72	28	42	142	9	7	11	27
50–54	98	47	49	194	16	9	23	48
55–59	153	59	70	282	22	9	21	52
60–64	163	50	58	271	22	14	22	58
65–69	103	48	65	216	25	12	27	64
70–74	72	29	62	163	17	17	23	57
75–79	40	10	32	82	10	11	18	39
80–84	18	6	11	35	11	2	6	19
85–	4	3	2	9	4	2	1	7
Total	761	309	428	1498	141	90	169	400

Fig. 5 represents mortality rates at various ages for lung cancer among men in Copenhagen arranged in quinquennial cohorts born in the period about the years given in the diagram.

It would seem reasonable to assume that the carcinogenic influences determining the incidence of cancer of the lung, whether they be of customary, occupational or hormonal character, will not begin before the age of 15 or perhaps a few years later. It appears from the tables that the cohorts of highest mortality at an early age do not show an increase in rates until after the 35th year of age. Consequently it will be justifiable to assume a minimal period of about 20 years from the beginning of the influence to the death of the patient from cancer of the lung under the conditions usual in Copenhagen. It follows logically that if we assume a later or earlier beginning of the carcinogenic influence we must respectively shorten or prolong the assumed period with a corresponding number of years. It should also be remembered that we must allow for wide variations either way.

Under these assumptions and from the observations illustrated in Fig. 2 and 3 it follows that the increase in mortality from cancer of the lung in Copenhagen beginning in 1931 must have been determined by a carcinogenic influence beginning not later than 1910.

It appears from Fig. 5 that the graphs for the quinquennial cohorts do not coincide as they would for most other sites of cancer, but are moving to the left, although now at a decreasing pace. This would not be explained by assuming that all cohorts were from a certain age subject to some carcinogenic influence unless we add that the influence was strengthened at some point of time, or during a limited period of years.

Now it is seen that the result of the carcinogenic influence increased particularly for men born between 1875 and 1885, while the effect on the following cohorts progressed at a slower rate. Under the assumption that the effect takes place from an age of about 15 to 20 years, and considering that the effect on the cohort born about 1885 must have been considerably stronger than for the previous cohorts, we are led to the assumption that the full effect must have been

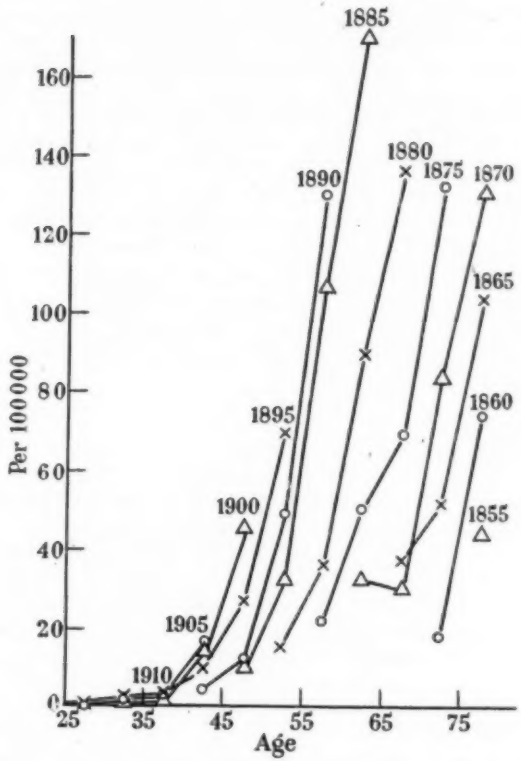

FIG. 5.—Cancer of the lung in Copenhagen, 1931 to 1950. Males. Mortality rates at various ages for quinquennial cohorts born about the year indicated.

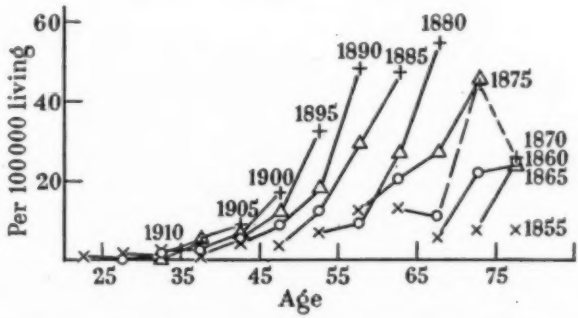

FIG. 6.—Cancer of the lung in Denmark. Provincial towns, 1931 to 1950. Males. Mortality rates at various ages for quinquennial cohorts born about the year indicated.

in action not earlier than 1900, although a slighter increase of the effect must have affected the following cohorts.

On the other hand the Cohort 1860 which showed some increase in mortality from cancer of the lung before they arrived at the age of 75 must have been exposed to the carcinogen about 20 to 25 years before they reached this age, which means that the exposure should have begun not later than 1910.

From these considerations it seems a fair assumption that the carcinogenic factor causing the increase of cancer of the lung in Copenhagen began to exert its full—or nearly full—effect between 1900 and 1910, although it would be a mistake not to allow for rather wide deviations either way.

A corresponding study of graphs for cohorts in provincial towns shows that the major displacement of the graphs apparently extended to younger cohorts than in the capital, so that the position of the graph for Cohort 1890 or perhaps 1895 takes almost the same relative position in the diagram as did the cohort of 1885 in the diagram for the capital.

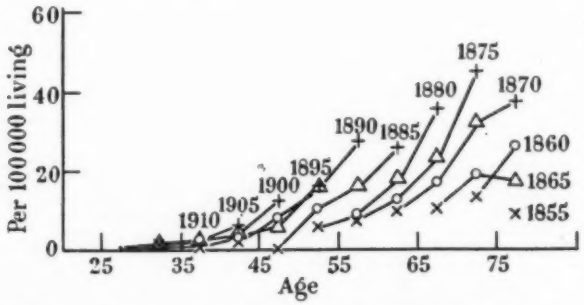

FIG. 7.—Cancer of the lung in Denmark. Rural areas, 1931 to 1950. Males. Mortality rates at various ages for quinquennial cohorts born about the year indicated.

The diagram for rural areas shows similar tendencies, although the difference from the provincial towns is doubtful. However, the graphs of this diagram have not yet taken the more vertical direction found in the other diagrams, so that they do not allow definite conclusions.

Returning to the diagram of Fig. 2, it will be seen that the differences in the level of the graphs indicating crude mortality rates for cancer of the lung between capital, provincial towns and rural areas can be ascribed to a later beginning of the rise in the latter categories, i.e., 8 years for provincial towns and 10 for the rural areas, which is in full conformity with the result of the cohort analysis.

Hence it appears that the assumption of atmospheric pollution as a cause of cancer of the lung in Denmark in order to explain the differences between towns and country is unnecessary, if we suppose that the carcinogenic influence began a few years later in the provincial towns and a few years later still in the country.

Quite apart from the etiological implications of our cohort study it is in the field of practical hygiene we find the widest consequences of the diagram of Fig. 6 when we apply the age distribution curve of the cohorts to the total male population. Even if we assume that the carcinogenic influence to which the Cohort 1905 was exposed represents the maximum possible, and that the age distribution

for men in Copenhagen does not change—both of which assumptions may be rather conservative—we find a heavy increase in the numbers of deaths from cancer of the lung in future Copenhagen as shown in Table IV.

TABLE IV.—*Future Annual Numbers of Deaths among Men in Copenhagen Computed on the Basis of the Age Distribution and Mortality of* 1946 *to* 1950 *and on the Age Distribution of Cancer of the Lung in Various Cohorts.*

1951–55	241	1971–75	808
1956–60	362	1976–80	919
1961–65	507	1981–85	979
1966–70	660	1986–90	1007

While the deaths among men from cancer of all sites for Greater Copenhagen in 1950 amounted to 852, of which 168 were ascribed to cancer of the lung, the figure for the latter category for 1990 amounts to about one thousand.

Is it likely that for the reduction of these figures cure will be better than prevention ?

SUMMARY.

The increase in mortality from cancer of the lung among men in Denmark has been demonstrated to be mainly due to a real increase in incidence of the disease, although a slight accentuation of the increase during the years following 1940, which can be demonstrated for both sexes, must be explained as due to improvements in diagnostic means.

An analysis of the increase in mortality rates on the basis of cohorts shows that the differences in crude mortality rates for cancer of the lung between Copenhagen, provincial towns and rural areas may be ascribed to a delay in onset of the carcinogenic influence of about 8 years for provincial towns and about 10 years for rural areas. Thus there is no reason to assume any carcinogenic influence of atmospheric pollution as far as Denmark is concerned.

Under the assumption that the carcinogenic influence does not begin earlier than about the 15th year of age or a few years later, the authors are led to believe that under conditions as in Copenhagen, it takes at least about 20 years from the beginning of the exposure to the carcinogenic effect till the death of the patient.

Under these assumptions the carcinogenic influence in Copenhagen must have begun to exert almost full influence about the period 1900 to 1910.

Our thanks are due to Knud Winge, M.D., Chief Physician of the Central Tuberculosis Station of Copenhagen, and to the Chief Statistician to the National Health Service, Kontorchef Marie Lindhardt, B.P.Sc., for kind permission to use material from their offices.

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THE INCIDENCE OF CANCER OF THE LUNG IN COAL MINERS IN ENGLAND AND WALES.

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THIS investigation was planned at a meeting on August 29, 1949, at the Baggeridge Colliery, Staffordshire, arranged by Dr. A. G. Marshall, now of the Royal Hospital, Wolverhampton, at which the following officers of the National Coal Board (West Midlands Division) were present: Dr. T. K. Elliott (Divisional Medical Officer); Mr. W. E. Raybould (Divisional Chief Scientist); Mr. W. Kirby (Divisional Safety Engineer), and Mr. A. Ray (South Staffordshire and Shropshire Area Chief Engineer).

Previous Investigations.

The incidence of cancer of the lung upon the coal miner is of peculiar interest because during his work he cannot smoke (except in about 10 per cent of pits), is withdrawn from sunlight, and inhales certain kinds of dust. In two earlier publications in this series (Kennaway and Kennaway, 1936, 1947) the low incidence of cancer of the lung, and of the larynx, upon coal miners has been recorded and their resemblance in this respect to agricultural workers has been pointed out (Table I). Cancer of the larynx is certainly not affected by all the factors which influence cancer of the lung (Kennaway and Kennaway, 1951); the similarity shown in this table is therefore noteworthy.

Some of the literature on the question, whether silicosis affects liability to cancer of the lung in any way, has been summarised in an earlier paper (Kennaway and Kennaway, 1947). Some small series of cases suggest that silicosis may predispose to cancer of the lung, but the general indication of the literature is that silicosis is not active in this respect. The present inquiry deals with the question, amongst others, whether the pneumokoniosis of coal miners affects the incidence of cancer of the lung.

Method of Present Investigation.

The death certificates of coal miners dying of cancer of the lung during the years 1937 to 1946 inclusive were taken from those for the whole male population of England and Wales, of which we receive copies through the kindness of the Registrar-General. These were classified—

(1) According to occupation, into those referring to (*a*) Face workers (code numbers 041, 042) and (*b*) Other workers below and above ground (see p. 15) and

(2) According to the Geographical Division in which the home of the deceased lay or, when this is not stated, the Division in which the death took place, these regions being the eight adopted by the National Coal Board (1950) (Table II).

The South-Western region includes the collieries of the Rhondda and adjacent valleys in Wales, and the small coalfields of the Forest of Dean and of the Bristol area. The smallest division, the South-Eastern, comprises four collieries only, in Kent. The data from this last field are omitted in some of the tables on account of the smallness of the numbers involved.

TABLE 1.—*Incidence of Cancer of the Lung and Larynx upon Coal Miners and Agricultural Workers.*

No comparison is attempted here of the two periods 1921–32 and 1933–38, which would require consideration of changes in age distribution.

Occupation.	Ratio of registered to 100 calculated deaths in males.			
	Cancer of lung.		Cancer of larynx.	
	1921–1932.	1933–1938.	1921–1932.	1933–1938.
General Population, England and Wales	100	100	100	100
Coal miners:				
Hewers and getters	63	72	58	73
Other workers below ground	44	46	44	45
Conveying material to shaft	38	47	44	42
Making roads	53	37	45	65
Workers above ground	71	47	55	68
Agricultural workers:				
Gardeners	58	61	75	79
Farmers	53	32	40	40
Agricultural labourers	34	26	41	60
Farm bailiffs and foremen	26	22	44	30

TABLE II.—*The Coalfields of England and Wales.* (*National Coal Board*).

Division.	Counties.	Average number of wage earners on colliery books, 1946.			
		All workers.	All workers underground.	Face workers.	Workers above ground.
Northern	Northumberland, Cumberland	45,811	34,190	17,008	11,621
Durham	Durham, Westmoreland	104,516	81,125	44,993	23,391
North-Eastern	Yorkshire	137,543	107,646	53,796	29,897
North-Western	Lancs., Cheshire, N. Wales	58,075	43,620	21,154	14,455
East Midlands	Derby, Notts., Northants, Lincs., Leics., Rutland	91,798	69,381	36,500	22,417
West Midlands	Salop, Staffs., Worcs., Warwick	58,914	43,701	20,777	15,213
South-Western	S. Wales, Glos., Somerset	114,856	92,601	48,392	22,255
South-Eastern	Kent	6,017	4,702	2,197	1,315
England and Wales		617,530	476,966	244,817	140,564
Per cent		100	77	40	23

Other workers underground, 232,149.

A Comparison of Seven Coalfields.

The deaths from cancer of the lung, in face-workers and others, in each of the seven coalfields and in each of the 10 years studied are recorded in Table III, and the numbers of these deaths in decennial age-groups ar given in Tables IV and V and in Fig. 1. The age distribution of the deaths from cancer of the lung in coal miners is not very different from that in the general population ; the age-group 50 to 69 contains, in round numbers, 65 per cent of the deaths in face-workers, 72 per cent of those in other coal miners, 68 per cent of those in all coal miners and 67·5 per cent of those in the general male population.

TABLE III.—*Deaths of Coal Miners. Cancer of Lung*, 1937–1946.

Age groups included are from 20 onwards. S.E. Coalfield is omitted.

	Face Workers (041, 042).								
	N.E.	W.M.	E.M.	S.W.	N.W.	D.	N.	Total.	
1937 . .	13	10	13	10	10	2	1	59	
1938 . .	19	4	15	8	7	2	5	60	
1939 . .	21	8	13	10	14	5	7	78	
1940 . .	19	7	7	12	11	6	4	66	
1941 . .	25	13	16	9	6	8	5	82	
1942 . .	16	7	8	13	9	10	8	71	
1943 . .	15	10	7	14	12	8	3	69	
1944 . .	21	2	10	17	16	13	3	82	
1945 . .	25	15	13	17	13	4	5	92	
1946 . .	20	10	14	14	15	12	5	90	
Total . .	194	86	116	124	113	70	46	749	

	Other Workers								Total coal miners.
1937 . .	9	3	2	5	7	7	2	35	94
1938 . .	17	2	8	5	6	9	4	51	111
1939 . .	9	4	7	7	7	5	10	49	127
1940 . .	7	8	11	13	3	11	9	62	128
1941 . .	14	5	5	10	6	7	6	53	135
1942 . .	8	4	12	5	8	13	5	55	126
1943 . .	12	2	7	8	8	9	8	54	123
1944 . .	24	9	5	15	7	14	7	81	163
1945 . .	19	5	10	18	10	18	12	92	184
1946 . .	31	11	19	16	7	17	14	115	205
Total . .	150	53	86	102	69	110	77	647	1396

A comparison of the incidence of cancer of the lung in the different coalfields has been attempted by dividing the numbers of employed (average of 1943–1946) by the numbers of deaths (1937–1946), and stating the result as the number of persons producing one death (Table VI and Fig. 2). Such a simple method can be used only if the age-distribution shows no great differences in the whole population of workers (Table VII and Fig. 3) ; the variations shown in Fig. 3 do not appear great enough to invalidate the comparison made in Fig. 2.

Pneumokoniosis.

The numbers of face-workers producing one death (Table VI and Fig. 2) show a three-fold range, from about 200 in N.W. to 600 in Durham, while the figure for S.W., which includes South Wales, is about 400 and is next to that for Durham, and the figure for non-face-workers in the S.W., namely 666, has the same relative

position in the series. Hence the area where there is most pneumokoniosis shows a low incidence of lung cancer.

The prevalence of pneumokoniosis in any area is indicated by the number of compensation cases arising under the Workmen's Compensation Acts and the Industrial Injuries Scheme. Some data, for which we are indebted to the Ministry of Fuel and Power (1951) are given in Table VIII.

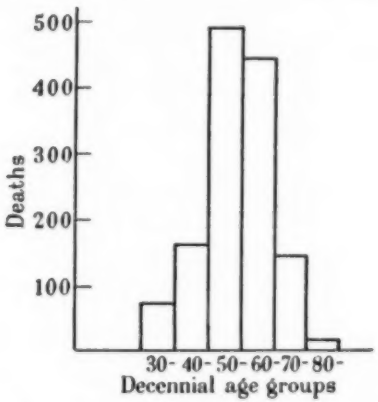

FIG. 1.—Deaths from Cancer of the Lung in Coal Miners in Decennial Age Groups. 1937–1946.

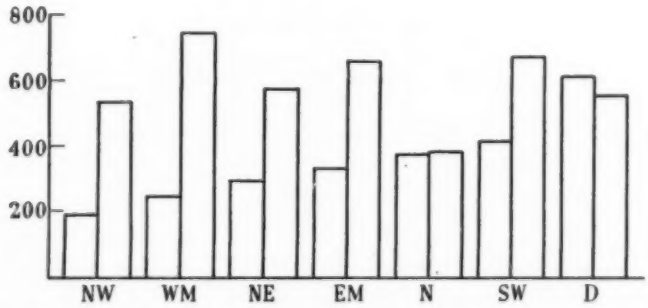

FIG. 2.—Numbers of Coal Miners producing one Death from Cancer of the Lung.
Left-hand columns: face-workers.
Right-hand columns: others.

Gooding (1946), in a study of pneumokoniosis in anthracite miners in South Wales, found no cases of lung cancer in 230 autopsies at which he was present. In others, making up with the above nearly 400 in all, there were three cases of primary cancer of the lung, namely (1) aged 68, refused certificate by Silicosis Medical Board; (2) aged 55, "fairly advanced silicosis"; and (3) aged 42, no fibrosis, died from accident.

TABLE IV.—*Coal Miners. Deaths from Cancer of Lung by Age-Groups. 1937–1946.*

Age groups included are from 30 onwards. S.E. Coalfield omitted.

Age at death. Decennial periods.	Number of deaths.		
	Face workers (041, 042).	Others.	Total.
30–	50	24	74
40–	114	98	212
50–	238	251	489
60–	237	208	445
70–	90	54	144
80–	13	4	17
Total	742	639	1381

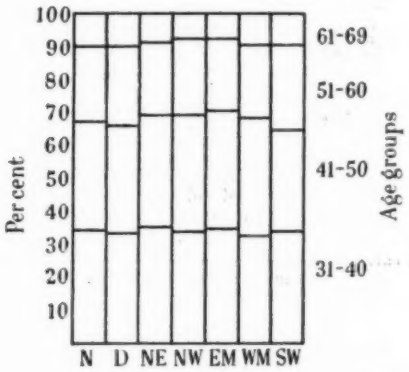

FIG. 3.—Percentage Age Distribution of Wage Earners on Colliery Books, December 11, 1948 (National Coal Board).

TABLE V.—*Percentage Distribution of Ages at Death from Cancer of Lung, 1937–1946.*

S.E. Coalfield included.

Age periods.	Coal miners.			General population. England and Wales. Males.
	Face workers (041, 042).	Others.	All.	
30–39	7·0	4·1	5·5	(25–39) 4·7
40–49	16·0	15·5	15·7	16·0
50–59	32·3 } 64·5	39·3 } 71·9	35·8 } 68·2	33·8 } 67·5
60–69	32·2	32·6	32·4	33·7
70–79	12·5	8·5	10·6	11·8
	100·0	100·0	100·0	100·0

Face-workers and Other Coal-workers.

In five of the seven coalfields represented in Table VI the incidence is greater on those at the face than on others, the largest difference, 3-fold, being in W.M., while in N. the figures are almost the same, and in D. the incidence upon the face-

workers is slightly lower. In S.W. the figure expressing the greater liability of face-workers, namely 1·6, is almost the same as that shown by all the coal-fields together (1·7, weighted mean 1·8)

TABLE VI.—*Numbers of Coal Miners producing One Death from Cancer of the Lung.*

Figures are in ascending order of numbers of face workers.

Division.	Face workers.	Others.	Ratio, Others : Face workers
N.W.	192	533	2·8
W.M.	243	733	3·0
N.E.	284	568	2·0
E.M.	323	655	2·0
N.	367	373	1·0
S.W.	409	666	1·6
D.	604	548	0·9
Mean	346	582	1·7
Weighted Mean	327	578	1·8

TABLE VII.—*Percentage Age-Distribution of Wage-earners (December 11, 1948). (National Coal Board.)*

Age-groups.	N.	Durham.	N.E.	N.W.	E.M.	W.M.	S.W.
31–40	34	33	35	33	34	32	33
41–50	33	33	34	36	36	36	31
51–60	23	24	22	23	22	22	26
61–69	10 } 33	10 } 34	9 } 31	8 } 31	8 } 30	10 } 32	10 } 36
	100	100	100	100	100	100	100

TABLE VIII.—*Regional Distribution of Pneumokoniosis*

Division (National Coal Board).	New cases of pneumokoniosis under the Industrial Injuries Act and the Workmen's Compensation Acts in 1951. (Ministry of Fuel and Power.)	Wage-earners on colliery books, 1950.	Rate per 1000.
Scotland	607	81,500	7·4
Durham	412	108,200	3·8
N.	88	48,600	1·8
N.E.	340	135,500	2·5
N.W.	276	57,500	4·8
E.M.	49	95,700	0·5
W.M.	388	56,200	6·9
S.E.	38	6,000	6·3
S.W.	1442	107,800	13·4
Total	3640	697,000	5·2
All Divisions other than S.W.	2198	589,200	3·7

The distinction between face-workers and other workers below ground is not always easy to define. The 'Classification of Occupations, 1950' (General Register Office, 1951) gives the following scheme:

Order III. Mining and Quarrying Occupations:

Sub-Order I: In Coal Mines.

040 Superintending Staff.
Workers below Ground.

041 Coal Cutting and Power-loading Machine Men.
042 Hewers and Getters (by hand).
043 Persons conveying Material to the Shaft.
044 Persons developing Underground Workings in Rock.
045 Persons repairing and maintaining Roads.
047 Other Workers below Ground.
049 Other Workers above Ground.

Sections 041 and 042 comprise 157 named occupations. The definition of face-worker, which class must include men working near, but not at, the actual face, varies in different coalfields, e.g., any man working within 20 yards of the

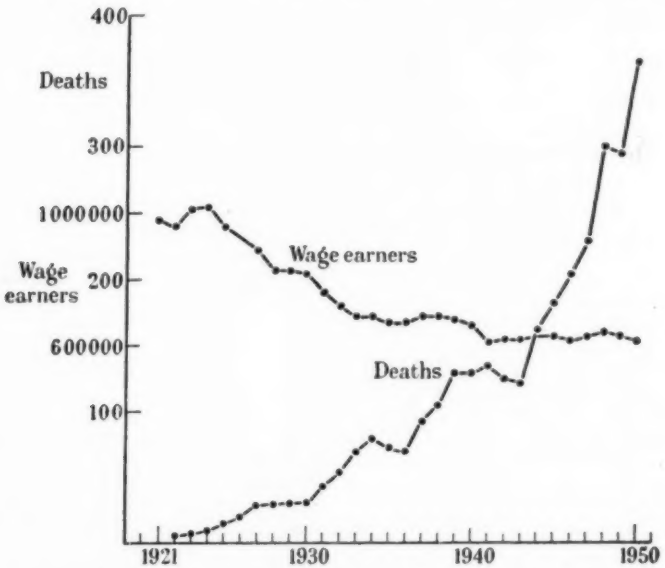

FIG. 4.—Numbers of Deaths from Cancer of the Lung in Coal Miners, and of Wage-Earners on Colliery Books, from 1921.

face may be included, but this criterion must be difficult to apply to non-stationary workers. However, in practice, the doubtful cases may not be very numerous, and one must match the entry on the death certificate as nearly as possible with one of the official occupations.

The Increase in Deaths from Cancer of the Lung.

The increase in deaths per annum attributed to cancer of the lung from 1921 to 1950 is shown in Tables III and IX and in Fig. 4. A satisfactory measure of this increase is difficult to find because the small numbers of deaths in the earlier years of course vary very widely and make the choice of an average difficult. However, the figures given indicate that the increase is similar in coal miners to that in other males. Moreover the figures in Table IX make no allowance for

the considerable fall in the numbers of wage-earners on colliery books from 978,500 in 1921 to 615,500 in 1950, the ratio of these figures being 1·6 : 1·0 (Table X and Fig. 4).

TABLE IX.—*Increase of Deaths attributed to Cancer of Lung in Coal Miners and in General Male Population.*

	Coal miners.			England and Wales.
	Face workers.	Others.	All.	Males.
1937	59	35	94	2914
1946	90	115	205	6765
Ratio	1 : 1·5	1 : 3·3	1 : 2·2	1 : 2·3

	Mean annual deaths.			Ratio.
	1921–1925.	1921–1930.	1946–1950.	
Coal miners	9·4	—	279	1 : 30·0
	—	18·9		1 : 14·8
England and Wales, Males	438	—	8,507	1 : 18·4
	—	615		1 : 13·8

TABLE X.—*Number of Wage-earners on Colliery Books, England and Wales. (National Coal Board.)*

Year.	Number.	Year.	Number.
1921	978,500	1936	670,300
1922	965,800	1937	688,300
1923	1,021,800	1938	691,700
1924	1,033,600	1939	678,000
1925	962,000	1940	662,800
1926	N/A	1941	614,700
1927	891,800	1942	625,600
1928	827,500	1943	625,000(*a*)
1929	834,800	1944	628,500
1930	821,600	1945	628,600
1931	763,000	1946	617,500(*b*)
1932	721,200	1947	630,300
1933	692,400	1948	641,600
1934	691,900	1949	636,800
1935	675,800	1950	615,500

(*a*) A new form of return led to a small increase in the total number of wage-earners employed.

(*b*) A standard method of recording wage-earners on books led to a small decline in the total number employed.

DISCUSSION.

(1) In earlier publications (Kennaway and Kennaway, 1936, 1947) attention was drawn to the low incidence of cancer of the lung in coal miners and to their resemblance in this respect to agricultural workers. "The air in a coal mine is pumped in from the outside, and collieries are not always situated in urban districts." Recently Stocks (1952) has extended his earlier demonstrations of the association of urban conditions with a higher incidence of cancer of the lung. In view of the investigations of Doll and Hill (1950) and others, the prohibition of smoking during the working day of miners underground must of course be considered. However, the incidence upon workers above ground, of whom the great majority can smoke, is also low (Table I); we have no information about their smoking habits.

(2) The peculiarities of the coal miner's life make the rapid increase of cancer of the lung (Tables III and IX and Fig. 4) of especial interest, and one hopes that this datum may encourage further inquiry; one might suggest that formerly pipe-smoking was the predominant custom among them, and hence the adoption of cigarettes (Doll and Hill, 1950, 1952) has had an especially distinct effect.

(3) The low incidence of cancer of the lung in coal miners shows that the inverse relationship between sunshine and this form of cancer (Stocks, 1947) is not due to any protective action exerted through the skin.

(4) The prevalence of pneumokoniosis in South Wales might be expected to eliminate many workers before they reach the age at which cancer of the lung is most frequent, and hence to be the cause of the low incidence; but actually men aged 50 to 69 are slightly more numerous in this coalfield (Table VII and Fig. 3.)

(5) The differences indicated in this paper between the different coalfields and between workers at the face and others (Table VI and Fig. 2) may be affected by a great variety of factors and cannot yet be based upon very large numbers. One hopes that these suggestions will be considered by those who have first-hand knowledge of the conditions in coal mines.

SUMMARY.

(1) The incidence of cancer of the lung in coal miners is less than that in the general population, and is similar to that upon agricultural workers. Some possible reasons for these differences are discussed.

(2) In the last 30 years the mortality from cancer of the lung has increased in coal miners in the same way as in the general population; in view of the peculiarities of the miner's life, this fact calls for further inquiry.

(3) The incidence of cancer of the lung differs (*a*) in face-workers and in other coal miners, and (*b*) in the various coalfields of England and Wales. It is low in the South Wales coalfield, where pneumokoniosis is most prevalent.

We are indebted to the officers of the National Coal Board named at the beginning of this paper for the suggestions which initiated this inquiry. We are very grateful to Mr. R. F. George of the National Coal Board for data and advice about matters dealt with in this paper. We wish to thank Dr. W. P. D. Logan and P. A. Phillips of the General Register Office for advice upon various matters and the Ministry of Fuel and Power for information about the incidence of pneumokoniosis in the various coalfields. This investigation has been supported by generous grants from the British Empire Cancer Campaign and the Anna Fuller Fund.

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THE RELATION BETWEEN DELAY IN TREATMENT OF CANCER AND SURVIVAL RATE.

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It has for long been an accepted principle that the earlier the diagnosis of malignant disease is made the more likely it is that the growth, if in a region of the body accessible to the surgeon, will be in a stage which admits of radical resection and the higher will be the survival rate. However, Balfour (1937) recorded that the 5-year survival rate for 2112 patients after resection of the stomach for carcinoma was 25 per cent for those patients with histories of symptoms for less than 6 months and 35 per cent for those with histories of symptoms for more than one year. Walters, Gray and Priestley (1942) in a later study recorded that 24·6 per cent of patients whose symptoms were of less than one year's duration survived 5 years after resection of the stomach for carcinoma, whereas 32·6 per cent of patients the duration of whose symptoms was more than one year survived 5 years. Swynnerton and Truelove (1952) reported on 375 patients with carcinoma of the stomach, 114 of whom were treated by resection; they found that the 3-year survival rate of 26 whose symptoms were of less than 6 months' duration was 7·7 per cent, of 22 whose symptoms were of 6 months' to 2 years' duration was 36·4 per cent, while for 24 whose symptoms were of over 2 years' duration the survival rate was 54·2 per cent. These differences in survival rate were found to be statistically significant ($P < 0{\cdot}01$). After an analysis of the age distribution of the patients, the site of the lesion and the possibility that the patients with long histories were examples of "ulcer-cancer", the authors concluded that these factors "do not serve to explain the good prognosis of patients with a long history in the resected group, and we believe that this association is due to the occurrence of slow-growing carcinomata". The authors investigated the association between the length of history and the grade of malignancy as judged by Broders' classification, and give a table which shows that 39 of the 114 patients had growths which were classified as Broders' Groups I and II, while 75 had growths classified as Broders' Groups III and IV; in each case the proportion of patients who gave histories of symptoms for more than 2 years was 23 per cent.

Eggers, de Cholnoky and Jessop (1941) reported that while the 5-year survivals of 235 patients who had undergone radical mastectomy for cancer of the breast fell steadily from 76 per cent to 20 per cent with delay in treatment up to 2 years, the survival rates rose to 25 per cent of 20 patients in whom the delay was over 2 years, and to 40·9 per cent of 22 patients in whose cases there had been delay of 3 years or over. Macdonald (1942), reporting on 1944 radical mastec-

tomies for cancer of the breast, found that 24·8 per cent of the 938 5-year survivors were treated more than one year after recognition of the tumour; he considered that the majority of these cases were the indolent, slowly-growing types of breast carcinoma, which metastasized late or occurred in hosts whose desmoplastic reaction to the tumour was intense. Bloom (1950*a*) in an analysis of 470 cases of cancer of the breast treated by surgery alone or with ancillary radiotherapy found, after classifying them into 3 groups according to histological criteria of degree of malignancy, that 79 per cent of 141 cases graded as of low malignancy survived 5 years, 42 per cent of 191 graded as of moderate malignancy and 25 per cent of 138 graded as of high malignancy; when the histological grading was subdivided according to the presence or absence of invasion of the axillary lymph nodes, he found that with lymph nodes not invaded the 5-year survival rates for Grades I, II and III were 94, 61 and 53 per cent respectively, while for patients in whom the lymph nodes were demonstrated histologically to be invaded the survival rates for the three grades were 65, 30 and 16 per cent respectively. Investigating the relationship between duration of symptoms and prognosis according to the grade of malignancy in the same series of cases, Bloom (1950*b*) found that of the patients with Grade I tumours 92 per cent of those attending hospital within 6 weeks of the first symptom survived 5 years, compared with 72 per cent of those who had waited 6 to 12 months and 78 per cent of those who had delayed more than 12 months. Those with Grade II tumours showed a survival rate of 41 to 47 per cent for periods of delay of up to 12 months, rising sharply to 50 per cent for those who had delayed more than 12 months, while for Grade III cases the survival rates fell from 29 per cent for those with only 6 weeks' delay to 21 per cent for those with more than 12 months' delay. It was found that 56 per cent of the patients with Grade I tumours came to hospital within 6 months and 23 per cent delayed more than 12 months, whereas for Grade III tumours the figures were 69 per cent and 10 per cent, showing that the most malignant neoplasms were associated with the shortest history and the patients with these tumours consulted their doctors earlier.

Park and Lees (1951) discussed in general terms the influence on survival rates of patients with cancer of the breast of variations in growth rate of individual tumours, infiltrating and metastasising potential, date of onset of symptoms, available time margin between the date when a tumour first became diagnosable and when it gave rise to regional and later incurable general metastases. They concluded that there was no direct evidence that delay in treatment diminished the cure rate, that the difference in 5-year survival rate between treated and untreated patients with cancer of the breast was about 20 per cent, that the difference between those operated on at the time of discovery of the tumour and those in whom there was delay of 3 years was 7·5 per cent, and that apparent curability can be explained in terms of variability of growth rate. Proof of curability will be given by a positive relation between decrease in survival rate and increased delay in treatment for cancers of the same degree of malignancy, from which it follows that the more rapidly growing the tumour the less the margin of time available for successful treatment and the greater the diminution of survival rate for a given time-delay. In an editorial comment the *British Medical Journal* (1952) remarks that Park and Lees "have made a strong case for attributing much of the benefit customarily allowed to surgery to the selection of patients with a naturally good prognosis."

In the analysis of 14,182 primary cases of cancer collected from London Hospitals by the Clinical Cancer Research Committee of the British Empire Cancer Campaign, Harnett (1952) gives for each region of the body the interval from first symptom to first consulting a doctor, which was over 6 months for 20·8 per cent of all patients, and the duration of symptoms at the time of commencing treatment, with comparison of the survival rates of those in whose cases it was over or under 6 months respectively. As all types of treatment, surgery, radiotherapy and combined treatment, whether radical or palliative, were included, it is not surprising that few significant differences in survival rate were detected, though in most regions it appeared that the patients treated early showed little better survival rates than those who came late.

In the present communication tabulations are presented from the same series of cases of the results of treatment of patients in Stages I, II or III, who were treated by radical surgery alone or combined with radiotherapy, arranged according to duration of symptoms at the time of commencing treatment. By the elimination of all patients who were in Stage IV, and of those in whose cases only operations of a palliative nature were performed, the series is restricted to those in whom there was a reasonable expectation of 5-year cure. Patients treated by radiotherapy alone have been excluded, because it is difficult to separate those in whose cases the treatment was intended to be curative from those in which it was palliative only, though some of the latter survived 5 years. The cases are grouped by site of the disease and by duration of symptoms at the time of commencing treatment into (*a*), those in which the interval from first symptom to commencement of treatment was less than 6 months, (*b*), those in which it was between 6 and 12 months, (*c*), those in which it was more than 12 months, (*d*), those in which it was unknown. The 5-year survival rates for each of these groups is expressed as a percentage of the total in the group, and the standard errors of the differences have been calculated to test for statistical significance. In the more important sites the survivals are shown by stages :

I. Confined to organ or tissue of origin, lymph nodes not involved.
II. Adjacent tissues invaded, but lymph nodes not involved clinically.
III. Lymph nodes involved clinically, with or without invasion of adjacent tissues.

In certain regions, stomach, kidney, testis, penis, breast and sarcoma of soft tissue, it is customary to classify cases with involvement of the regional lymph nodes as Stage II and those with invasion of adjacent tissues as Stage III ; allowance has been made for this difference when necessary.

It was found that in some regions the highest percentage of 5-year survivals was among those patients who came for treatment in the first 6 months, in others the highest percentage was among those who came in the second 6 months, and in others it was among those who came after symptoms had been noticed for more than one year. Accordingly the cases have been arranged in three groups (Tables I–VII).

For the whole group of 1097 cases of known duration the difference between those treated in the first 6 months and those treated later is the difference between survival rates of 51·1 per cent and of 42·0 per cent, which is 9·1 per cent. Detailed figures of survival by stages are given in Table II for squamous carcinoma of the skin, corpus uteri, female breast and kidney ; for the other regions in the group all stages are combined.

A. *The highest percentage of 5-year survivals was among the patients who were treated within the first 6 months.*

TABLE I.

Site.	0–6 months.		6–12 months.		Over 12 months.		Not known.	
	No.	Survd.	No.	Survd.	No.	Survd.	No.	Survd.
Skin, squamous carcinoma	55	38	22	5	42	23	19	5
Corpus uteri	62	39	46	24	51	28	6	2
Penis	17	10	7	4	6	1	1	–
Cervix uteri	43	24	17	7	9	4	2	2
Female breast	346	171	84	35	111	51	17	6
Kidney	23	11	10	1	12	4	1	–
Bladder, papillary carcinoma	56	20	21	7	33	7	1	–
Pharynx	14	2	4	–	6	1	1	–
Totals	616	315	211	83	270	119	48	15
Per cent survived	51·1		39·3		44·1		31·2	
Difference	11·8		4·8					

TABLE II.

Skin—squamous carcinoma.

Duration.	Stage I.		Stage II.		Stage III.		Totals.		Per cent.	Difference.
	No.	Survd.	No.	Survd.	No.	Survd.	No.	Survd.		
0–3 months	25	21	4	2	4	2	33	25	69·1±6·2	46·4 ±10·9
3–6 „	19	12	2	1	1	–	22	13		
6–12 „	18	4	2	1	2	–	22	5	22·7±8·9	32·1 ±9·9
Over 12 „	29	20	7	1	6	2	42	23	54·8±7·7	
Not known	9	3	5	2	5	–	19	5		

Both the above differences in survival rate are statistically significant.
For cases of known duration $\chi^2=13\cdot69$, $n=2$, $P<\cdot01>\cdot001$.

Corpus uteri.

Duration.	Stage I.		Stage II.		Stage III.		Totals.		Per cent.	Difference.
	No.	Survd.	No.	Survd.	No.	Survd.	No.	Survd.		
0–3 months	26	18	3	1	2	1	31	20	62·9±6·1	10·7 ±9·6
3–6 „	24	17	2	1	5	1	31	19		
6–12 „	35	19	8	4	3	1	46	24	52·2±7·3	2·7 ±11·9
Over 12 „	37	19	13	8	1	1	51	28	54·9±7·0	
Not known	5	2	1	–	–	–	6	2		

The differences in survival rate are not statistically significant.
For cases of known duration $\chi^2=1\cdot41$, $n=2$, $P<\cdot50>\cdot30$.

Female breast.

Duration.	Stage I.		Stage II.		Stage III.		Totals.		Per cent.	Difference.
	No.	Survd.	No.	Survd.	No.	Survd.	No.	Survd.		
0–3 months	79	44	87	44	53	21	219	109	49·4±2·7	7·7 ±6·0
3–6 „	30	22	45	16	52	24	127	62		
6–12 „	26	15	25	12	33	8	84	35	41·7±5·4	4·2 ±7·2
Over 12 „	30	19	19	7	62	25	111	51	45·9±4·7	
Not known	8	3	6	2	3	1	17	6		

The differences in survival rate are not statistically significant.
For cases of known duration $\chi^2=1\cdot77$, $n=2$, $P<\cdot50>\cdot30$.

TABLE II—*continued.*

Kidney.

Duration.	Stage I. No.	Stage I. Survd.	Stage II. No.	Stage II. Survd.	Stage III. No.	Stage III. Survd.	Totals. No.	Totals. Survd.	Per cent.	Difference.
0–3 months	7	3	1	1	5	1	13	5	47·8±10·4	37·8 ±14·1
3–6 „	5	3	–	–	5	3	10	6		
6–12 „	8	1	–	–	2	–	10	1	10·0± 9·5	23·3 ±16·6
Over 12 „	10	3	1	–	1	1	12	4	33·3±13·6	
Not known	1	–	–	–	–	–	1	–		

The difference in survival rate between those of duration 0–6 months and those of duration 6–12 months, 37·8±14·1, is statistically significant.
For cases of known duration $\chi^2=4\cdot40$, $n=2$, $P<\cdot20>\cdot10$.

Penis.

Duration.	All stages. No.	All stages. Survd.	Per cent.	Difference.
0–3 months	12	8	58·8±11·9	1·7±22·1
3–6 „	5	2		
6–12 „	7	4	57·1±18·7	40·5±24·1
Over 12 „	6	1	16·6±15·2	
Not known	1	–		

The differences in survival rate are not statistically significant.
For cases of known duration $\chi^2=3\cdot32$, $n=2$, $P<\cdot20>\cdot10$.

Cervix uteri.

Duration.	All stages. No.	All stages. Survd.	Per cent.	Difference.
0–6 months	43	24	55·8± 7·6	14·6±14·1
6–12 „	17	7	41·2±11·9	3·2±20·4
Over 12 „	9	4	44·4±16·5	
Not known	2	2		

The differences in survival rate are not statistically significant.
For cases of known duration $\chi^2=1\cdot20$, $n=2$, $P<\cdot70>\cdot50$.

Bladder—papillary carcinoma.

Duration.	All stages. No.	All stages. Survd.	Per cent.	Difference.
0–6 months	56	20	35·7± 6·4	2·4±12·1
6–12 „	21	7	33·3±10·3	12·1±12·5
Over 12 „	33	7	21·2± 7·1	
Not known	1	–		

The differences in survival rate are not statistically significant.
For cases of known duration $\chi^2=2\cdot12$, $n=2$, $P<\cdot50>\cdot30$.

Pharynx.

Duration.	All stages. No.	All stages. Survd.	Per cent.	Difference.
0–6 months	14	2	14·3±9·3	4·3±13·3
6–12 „	4	–	10·0±9·5	
Over 12 „	6	1		
Not known	1	–		

The differences in survival rate are not statistically significant.
For cases of known duration $\chi^2=\cdot102$, $n=1$, $P<\cdot80>\cdot70$.

All cases of cancer of the female breast in Stages I, II and III are included in this table, whether treated by radical mastectomy alone or combined with radiotherapy. The proportions in the three stages are 31·0, 32·6 and 36·4 per

cent respectively. Among 383 patients treated by radical mastectomy alone, 35·5 per cent were in Stage I and the best survival rate for all stages combined was 50·9 ± 3·3 per cent for those treated during the first 6 months; among 175 who were treated by radical mastectomy combined with radiotherapy only 21·1 per cent were in Stage I and 43·4 per cent were in Stage III, and the best survival rate for all stages combined was 58·8 ± 8·4 per cent for patients treated one year or more subsequent to the first symptom, who numbered 34. The difference of 7·9 ± 9·0 between these survival rates is not statistically significant.

When the sites were subdivided into those in which the highest survival rates were among the patients who came under treatment in less than 3 months and those in which the highest rates were among those treated in the second 3 months, it was found that the former included squamous carcinoma of the skin, corpus uteri, penis and female breast with a survival rate for all groups of 54·9 per cent, while the latter included cervix uteri, kidney, papillary carcinoma of the bladder and carcinoma of the pharynx (treated by radical surgery or by combined methods) with a 5-year survival rate for all groups of 51·0 per cent.

The age distribution of 702 patients with cancer of the breast who were treated by radical mastectomy alone, of 393 who were treated by radical mastectomy combined with radiotherapy, of 80 patients with cancer of the corpus uteri treated by panhysterectomy and of 95 with cancer of the cervix uteri treated by Wertheim's hysterectomy were investigated by Harnett (1948, 1949); in addition that of 138 patients with squamous carcinoma of the skin and of 40 with carcinoma or hypernephroma of the kidney have been tested by the χ^2 test, but in no case were there any significant differences between the observed and expected survivals in the different age groups.

When the 616 patients who came for treatment within the first 6 months were classified according to the stage of the disease at the time of commencing treatment, it was found that 46·6 per cent were in Stage I, in which the disease is still local, and 53·4 per cent were in later stages with involvement of regional lymph nodes or invasion of adjacent tissues or both.

B. *The highest percentage of 5-year survivals was among the patients who were treated from 6 to 12 months after the first symptom.*

TABLE III.

Site.	0–6 months.		6–12 months.		Over 12 months.		Not known.	
	No.	Survd.	No.	Survd.	No.	Survd.	No.	Survd.
Salivary glands	5	4	2	2	19	13	1	–
Tonsil	13	3	1	1	4	2	–	–
Testis	19	10	4	3	8	6	1	1
Colon, all sites	187	63	34	12	63	20	13	3
Skin, malignant melanoma	14	3	9	3	12	2	2	–
Nasal sinuses	25	5	8	2	3	–	2	1
Bladder, infiltrating carcinoma	38	5	14	3	13	2	2	–
Stomach	98	11	62	15	63	9	18	2
Totals	399	104	134	41	185	54	39	7
Per cent survived	26·1		30·6		29·2		18·0	
Difference	4·5			1·4				

Detailed figures of survival by stages are given in Table IV for the colon (all sites) and the stomach; for the other regions in the group all stages are combined.

TABLE IV.

Colon (all sites).

Duration.	Stage I. No.	Stage I. Survd.	Stage II. No.	Stage II. Survd.	Stage III. No.	Stage III. Survd.	Totals. No.	Totals. Survd.	Per cent.	Difference.
0–6 months	105	39	35	12	47	12	187	63	33·7±3·4	1·6 ±8·9
6–12 „	15	6	12	4	7	2	34	12	35·3±8·2	
Over 12 „	31	10	19	5	13	5	63	20	31·7±5·9	3·6 ±10·1
Not known	7	3	3	–	3	–	13	3		

The differences in survival rate are not statistically significant.
For cases of known duration $\chi^2 = \cdot 135$, $n=2$, $P < \cdot 95 > \cdot 90$.

Stomach (all sites).

Duration.	Stage I. No.	Stage I. Survd.	Stage II. No.	Stage II. Survd.	Stage III. No.	Stage III. Survd.	Totals. No.	Totals. Survd.	Per cent.	Difference.
0–6 months	38	7	50	2	10	2	98	11	11·2±3·2	13·0 ±6·3
6–12 „	25	11	30	3	7	1	62	15	24·2±5·4	
Over 12 „	23	5	35	4	5	–	63	9	14·3±4·4	9·9 ±7·0
Not known	8	–	6	2	4	–	18	2		

The difference between the survival rates of those operated on within 6 months and those operated on in the second 6 months from the first symptom, $13 \pm 6 \cdot 3$, is statistically significant.
For cases of known duration $\chi^2 = 4 \cdot 95$, $n=2$, $P < \cdot 10 > \cdot 05$.

Salivary glands.

Duration.	All stages. No.	All stages. Survd.	Per cent.	Difference.
0–6 months	5	4	80·0±17·9	8·6±20·4
6–12 „	2	2	71·4± 9·8	
Over 12 „	19	13		
Not known	1	–		

The difference is not statistically significant.
For cases of known duration $\chi^2 = \cdot 158$, $n=1$, $P < \cdot 70 > \cdot 50$.

Tonsil.

Duration.	All stages. No.	All stages. Survd.	Per cent.	Difference.
0–6 months	13	3	23·1±11·7	36·9±24·8
6–12 „	1	1	60·0±21·9	
Over 12 „	4	2		

The difference is not statistically significant.
For cases of known duration $\chi^2 = 2 \cdot 23$, $n=1$, $P < \cdot 20 > \cdot 10$.

Testis (excluding cases of teratoma).

Duration.	All stages. No.	All stages. Survd.	Per cent.	Difference.
0–6 months	19	10	52·6±11·4	22·4±17·0
6–12 „	4	3	75·0±12·5	
Over 12 „	8	6		
Not known	1	1		

The difference is not statistically significant.
For cases of known duration $\chi^2 = 1 \cdot 56$, $n=1$, $P < \cdot 30 > \cdot 20$.

TABLE IV—*continued*.

Skin—malignant melanoma.

Duration.	All stages. No.	Survd.	Per cent.	Difference.
0–6 months	14	3	21·4±10·9	11·9±19·2
6–12 „	9	3	33·3±15·7	16·7±19·0
Over 12 „	12	2	16·6±10·7	
Not known	2	–		

The differences in survival rate are not statistically significant.
For cases of known duration $\chi^2 = \cdot 834$, $n = 2$, $P < \cdot 70 > \cdot 50$.

Bladder—infiltrating carcinoma.

Duration.	All stages. No.	Survd.	Per cent.	Difference.
0–6 months	38	5	13·2± 5·5	8·2±12·2
6–12 „	14	3	21·4±10·9	6·0±14·8
Over 12 „	13	2	15·4±10·0	
Not known.	2	–		

The differences in survival rate are not statistically significant.
For cases of known duration $\chi^2 = \cdot 54$, $n = 2$, $P < \cdot 80 > \cdot 70$.

Nasal sinuses.

Duration.	All stages. No.	Survd.	Per cent.	Difference.
0–6 months	25	5	20·0± 8·0	1·8±14·1
6–12 „	8	2	18·2±11·6	
Over 12 „	3	–		
Not known	2	1		

The differences in survival rate are not statistically significant.
For cases of known duration $\chi^2 = \cdot 016$, $n = 1$, $P < \cdot 90 > \cdot 80$.

There are no significant differences between the survival rates of the three groups of patients with carcinoma of the colon, nor were any found when the figures for the different regions of the colon were examined separately. In the case of the stomach, however, the best results of operation were in the group of 62 patients treated in the second 6 months from first symptom, whose survival rate was 24·2 per cent against 11·2 per cent for 98 operated on within the first 6 months; the difference of $13 \cdot 0 \pm 6 \cdot 3$ is statistically significant. The group of 63 in whom the symptoms were of over 12 months' standing at the time of operation had a survival rate of 14·3 per cent; the difference of $9 \cdot 9 \pm 7 \cdot 0$ between this figure and that for the group treated in the second 6 months is not statistically significant. If the patients are grouped into those of less than 6 months' duration, with a survival rate of 11·2 per cent, and those of over 6 months' duration, with a survival rate of 19·2 per cent, the difference of $8 \cdot 0 \pm 4 \cdot 7$ is not significant.

The age distribution of the patients with cancer of the stomach and colon were investigated by the χ^2 test, but in neither case were there any significant differences between the observed and expected survivals in the different age groups; for the stomach cases $P < \cdot 20 > \cdot 10$ and for the colon cases $P < \cdot 70 > \cdot 50$, showing that the differences in survival rates were not due to differences in age distribution. The survival rate for cancer of the stomach was 20·4 per cent for 108 patients in the age groups 25–54 and 11·1 per cent for 133 patients of 55 and upwards, but the difference of $9 \cdot 3 \pm 4 \cdot 7$ is not significant, though the prognosis was not so good in the older patients. The 15 survivors of those

treated in the second 6 months were distributed evenly over the age-groups, with the greatest number in the 45–54 group.

When the 134 patients who came under treatment 6–12 months from the first symptom and had the best survival rate were classified according to the stage which the disease had reached when treatment was commenced, it was found that 39·6 per cent were still in Stage I and 60·4 per cent were in later stages.

C. *The highest percentage of 5-year survivals was among the patients who were treated 12 months or more subsequent to the first symptom.*

TABLE V.

Site.	0–6 months.		6–12 months.		Over 12 months.		Not known.	
	No.	Survd.	No.	Survd.	No.	Survd.	No.	Survd.
Intrinsic larynx	8	3	6	2	9	7	–	–
Thyroid gland	23	12	6	4	20	14	3	3
Vulva and female urethra	22	8	14	6	16	11	3	–
Lip, upper and lower	27	10	10	5	18	12	2	1
Bone sarcoma (all types)	27	10	7	4	8	5	1	–
Ovary, malignant papillary cysts	44	16	11	6	12	7	2	1
Sarcoma of soft tissue	35	17	4	2	30	17	3	1
Rectum, ampulla	137	42	70	22	61	31	11	–
Eye	32	12	8	4	10	5	7	3
Male breast	3	–	2	1	4	2	–	–
Recto-sigmoid	56	15	39	9	25	11	7	3
Tongue	47	15	9	3	14	6	–	–
Anal canal and anus	16	5	8	1	8	3	3	–
Ovary, carcinoma	37	6	6	–	8	3	3	1
Mouth, all sites	51	8	7	1	13	4	–	–
Prostate	19	3	8	2	10	3	1	–
Totals	584	182	215	72	266	141	46	13
Per cent survived	31·2		33·5		53·0			
Difference	2·3		19·5					

For the whole group of 1065 cases of known duration the survival rate of 799 patients who were treated within 12 months of the first symptom was 31·8 per cent, whereas for 266 who were treated later it was 53·0 per cent. Detailed figures of survival by stages are given below for the vulva, lip, ovary (malignant papillary cysts), ampulla of rectum, recto-sigmoid, tongue and mouth. For other regions all stages are combined.

TABLE VI.

Vulva and female urethra.

Duration.	Stage I.		Stage II.		Stage III.		Totals.		Per cent.	Difference.
	No.	Survd.	No.	Survd.	No.	Survd.	No.	Survd.		
0–6 months	14	8	1	–	7	–	22	8	36·4±10·2	6·5 ±16·7
6–12 „	10	5	–	–	4	1	14	6	42·9±13·2	
Over 12 „	11	8	1	1	4	2	16	11	68·7±11·6	25·8 ±17·6
Not known	–	–	–	–	3	–	3	–		

The differences in survival rate are not statistically significant.
For cases of known duration $\chi^2=4\cdot11$, $n=2$, $P<\cdot20>\cdot10$.

TABLE VI—*continued.*

Lip, upper and lower.

Duration.	Stage I. No.	Stage I. Survd.	Stage II. No.	Stage II. Survd.	Stage III. No.	Stage III. Survd.	Totals. No.	Totals. Survd.	Per cent.	Difference.
0–6 months	16	8	1	–	10	2	27	10	37·0± 9·3	13·0
6–12 „	4	2	–	–	6	3	10	5	50·0±15·8	±18·3
										16·6
Over 12 „	14	11	1	–	3	1	18	12	66·6±11·1	±19·3
Not known	2	1	–	–	–	–	2	1		

The differences in survival rate are not statistically significant.
For cases of known duration $\chi^2=3\cdot794$, $n=2$, $P<\cdot20>\cdot10$.

Ovary—malignant papillary cysts.

Duration.	Stage I. No.	Stage I. Survd.	Stage II. No.	Stage II. Survd.	Stage III. No.	Stage III. Survd.	Totals. No.	Totals. Survd.	Per cent.	Difference.
0–6 months	36	15	8	1	–	–	44	16	36·4± 7·2	18·1
6–12 „	9	5	2	1	–	–	11	6	54·5±15·0	±16·7
										3·8
Over 12 „	10	7	2	–	–	–	12	7	58·3±14·2	±20·7
Not known	2	1	–	–	–	–	2	1		

The differences in survival rate are not statistically significant.
For cases of known duration $\chi^2=2\cdot56$, $n=2$, $P<\cdot30>\cdot20$.

Rectum, ampulla.

Duration.	Stage I. No.	Stage I. Survd.	Stage II. No.	Stage II. Survd.	Stage III. No.	Stage III. Survd.	Totals. No.	Totals. Survd.	Per cent.	Difference.
0–6 months	40	19	44	14	53	9	137	42	30·7±3·9	0·7
6–12 „	22	9	25	8	23	5	70	22	31·4±5·6	±6·8
										19·4
Over 12 „	16	11	23	11	22	9	61	31	50·8±6·4	±8·5
Not known	6	–	3	–	2	–	11	–		

The difference of 19·4±8·5 per cent between the survival rate of those patients who were treated in the second 6 months and of those whose duration was more than 12 months is statistically significant.
For cases of known duration $\chi^2=8\cdot17$, $n=2$, $P<\cdot02>\cdot01$.

Recto-sigmoid.

Duration.	Stage I. No.	Stage I. Survd.	Stage II. No.	Stage II. Survd.	Stage III. No.	Stage III. Survd.	Totals. No.	Totals. Survd.	Per cent.	Difference.
0–6 months	15	3	17	6	24	6	56	15	26·8±5·9	3·7
6–12 „	13	4	10	4	16	1	39	9	23·1±6·7	±9·0
										20·9
Over 12 „	9	6	8	4	8	1	25	11	44·0±9·9	±12·0
Not known	3	2	4	1	–	–	7	3		

The differences in survival rate are not statistically significant.
For cases of known duration $\chi^2=3\cdot52$, $n=2$, $P<\cdot20>\cdot10$.

TABLE VI—*continued.*

Tongue.

Duration.	Stage I.		Stage II.		Stage III.		Totals.		Per cent.	Difference.
	No.	Survd.	No.	Survd.	No.	Survd.	No.	Survd.		
0–6 months	29	12	2	1	16	2	47	15	31·9±6·8	1·4 ±17·1
6–12 „	7	3	–	–	2	–	9	3	33·3±15·7	9·6 ±20·5
Over 12 „	9	5	–	–	5	1	14	6	42·9±13·2	

The differences in survival rate are not statistically significant.
For cases of known duration $\chi^2 = \cdot 585$, $n=2$, $P < \cdot 80 > \cdot 70$.

Mouth—all sites.

Duration	Stage I.		Stage II.		Stage III.		Totals.		Per cent.	Difference.
	No.	Survd.	No.	Survd.	No.	Survd.	No.	Survd.		
0–6 months	21	5	10	1	20	2	51	8	15·7± 5·1	1·4 ±14·2
6–12 „	3	1	–	–	4	–	7	1	14·3±13·2	28·6 ±18·7
Over 12 „	9	5	–	–	5	1	14	6	42·9±13·2	

The differences in survival rate are not statistically significant.
For cases of known duration $\chi^2 = 1 \cdot 65$, $n=2$, $P < \cdot 50 > \cdot 30$.

Intrinsic larynx.

Duration.	All stages.		Per cent.	Difference.
	No.	Survd.		
0–6 months	8	3	37·5±17·1	4·2±25·7
6–12 „	6	2	33·3±19·2	
Over 12 „	9	7	77·7±13·9	44·4±23·7

The differences in survival rate are not statistically significant.
For cases of known duration $\chi^2 = 3 \cdot 92$, $n=2$, $P < \cdot 20 > \cdot 10$.

Thyroid Gland,

Duration.	All stages.		Per cent.	Difference.
	No.	Survd.		
0–6 months	23	12	52·2±10·4	14·4±21·9
6–12 „	6	4	66·6±19·2	
Over 12 „	20	14	70·0±10·2	3·4±21·8
Not known	3	3		

The differences in survival rate are not statistically significant.
For cases of known duration $\chi^2 = 1 \cdot 55$, $n=2$, $P < \cdot 50 > \cdot 30$. If the 3 cases of unknown duration are included $\chi^2 = 3 \cdot 505$, $n=3$, $P < \cdot 50 > \cdot 30$.

Bone sarcoma (all types).

Duration.	All stages.		Per cent.	Difference.
	No.	Survd.		
0–6 months	27	10	37·0± 9·3	20·1±20·9
6–12 „	7	4	57·1±18·7	
Over 12 „	8	5	62·5±17·1	5·4±25·3
Not known	1	–		

The differences in survival rate are not statistically significant.
For cases of known duration $\chi^2 = 2 \cdot 09$, $n=2$, $P < \cdot 50 > \cdot 30$.

TABLE VI—*continued.*

Soft tissue sarcoma (all types).

Duration.	All stages. No.	Survd.	Per cent.	Difference.
0–6 months	35	17		
6–12 „	4	2	48·7±8·0	7·9±12·1
Over 12 „	30	17	56·6±9·0	
Not known	3	1		

The difference in survival rate is not statistically significant.
For cases of known duration $\chi^2 = \cdot 44$, $n = 1$, $P < \cdot 70 > \cdot 50$.

Eye (all types of malignant growth).

Duration.	All stages. No.	Survd.	Per cent.	Difference.
0–6 months	32	12	37·5± 8·5	12·5±19·6
6–12 „	8	4	50·0±17·7	0·0
Over 12 „	10	5	50·0±15·8	
Not known	7	3		

The difference in survival rate is not statistically significant.
For cases of known duration $\chi^2 = \cdot 73$, $n = 2$, $P < \cdot 70 > \cdot 50$.

Anal canal and anus.

Duration.	All stages. No.	Survd.	Per cent.	Difference.
0–6 months	16	5	31·2±11·6	18·7±16·4
6–12 „	8	1	12·5±11·7	25·0±20·7
Over 12 „	8	3	37·5±17·1	
Not known	2	–		

The differences in survival rate are not statistically significant.
For cases of known duration $\chi^2 = 1 \cdot 39$, $n = 2$, $P < \cdot 50 > \cdot 30$.

Ovary—carcinoma and sarcoma.

Duration.	All stages. No.	Survd.	Per cent.	Difference.
0–6 months	37	6	16·2± 6·0	21·3±18·1
6–12 „	6	–	0·0	
Over 12 „	8	3	37·5±17·1	
Not known	3	1		

The difference in survival rate is not statistically significant.
For cases of known duration $\chi^2 = 3 \cdot 51$, $n = 2$, $P < \cdot 20 > \cdot 10$.

Prostate.

Duration.	All stages. No.	Survd.	Per cent.	Difference.
0–6 months	19	3	15·8± 8·4	9·2±17·4
6–12 „	8	2	25·0±15·3	5·0±21·0
Over 12 „	10	3	30·0±14·5	
Not known	1	–		

The differences in survival rate are not statistically significant.
For cases of known duration $\chi^2 = \cdot 85$, $n = 2$, $P < \cdot 70 > \cdot 50$.

In all these sites the 5-year survival rates of those patients who came under treatment 12 months or more after the first symptom were better than those of the patients who were treated at an earlier stage, but the only difference which is statistically significant is between the survival rate of 50·8 per cent for 61

patients with cancer of the ampulla of the rectum of over 12 months' standing and 31·4 per cent for 70 patients whose disease was of 6–12 months' standing, difference 19·4 ± 8·3. When the survival rates for cancer of the ampulla of the rectum were tested by the χ^2 test the value of $P < \cdot 02 > \cdot 01$ was found, mainly due to the difference between the observed and expected survivals in the group of over 12 months' duration.

The age distribution of the patients with cancer of the recto-sigmoid and of those with cancer of the ampulla of the rectum were investigated by the χ^2 test, but in neither case were any significant differences found between the observed and expected survivals; for both regions $P < \cdot 90 > \cdot 80$, showing that the differences in survival rates were not due to differences in age distribution.

When the 266 patients who came under treatment more than 12 months from the first symptom and had the highest survival rate were classified according to the stage of the disease at the time of commencing treatment, it was found that 53·8 per cent were still in Stage I and 46·2 were in later stages, a higher percentage of Stage I cases than in either Group A or Group B.

In Table VII all three groups are combined:

TABLE VII.

Group.	Duration.	0–6 months.		6–12 months.		Over 12 months.		Not known.	
		No.	Survd.	No.	Survd.	No.	Survd.	No.	Survd.
A	0–6 months	616	315	211	83	270	119	48	15
B	6–12 „	399	104	134	41	185	54	39	7
C	Over 12 „	584	182	215	72	266	141	46	13
	Totals	1599	601	560	196	721	314	133	35
	Per cent survived	37·6		35·0		43·6		26·3	
	Difference		2·6		8·6				

TABLE VIII.—*Grand Totals of Cases of Known Duration.*

Duration.	No.	Survived.	Per cent.	Difference.
Under 12 months	2159	797	36·9	6·7
Over „	721	314	43·6	

The difference of survival rate between those who were treated within 6 months of the first symptom and those who were treated 6–12 months from the first symptom is small, but the survival rate of those who were treated after a delay of 12 months or more is greater than that of the other two groups, either combined or separately; numerically the latter group comprises about one-quarter of all cases. As has been stated above this difference cannot be accounted for by differences in the age distribution of patients in either of the groups, so it must be presumed to be due to variations in the rate of growth of individual tumours and in their infiltrating and metastatising powers. All cases of patients with distant metastases have been eliminated from the present series, which was confined to those in which the tumour was local or had given rise to clinically recognisable metastases in lymph nodes; they were selected as operable cases, no patient in an advanced stage or suitable only for palliative treatment being included. Small differences were found in the proportion of patients in Stage I among those with the highest survival rate in each of the three groups; for Group

A this was 46·6 per cent, for Group B it was 39·6 per cent and for Group C it was 53·8 per cent, but the combined figures for all the patients in each group showed only small deviations from the mean.

Classification by Stages of all Cases of Known Duration.

TABLE IX.

Stage.	A. 0–6 months.		B. 6–12 months.		C. Over 12 months.		Totals.	
	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.
I	526	47·9	323	45·0	550	51·6	1399	48·6
II	315	28·7	256	35·7	234	22·0	805	28·0
III	256	23·3	139	19·4	281	26·4	676	23·5
Totals	1097	99·9	718	100·1	1065	100·0	2880	100·1

The highest percentage of Stage I cases is among the patients whose disease was of over 12 months' standing (Group C), probably due, like the difference in survival rates between those treated early and those treated later, to variations in the intrinsic growth-rate of the tumours themselves. Park and Lees (1951) point out that this may be estimated by (*a*) the histological appearance of the tumour, disregarding size or degree of extension at the time of diagnosis, or (*b*) by duration of survival from the onset of symptoms or time of treatment to death in untreated or unsuccessfully treated cases, though in the latter case the survival time may be affected by the treatment.

Pathology.

Histological reports were available in 88 per cent of the 3013 cases. In 376 cases the histological grade of the growths according to Broders' classification was given, but as these reports were the work of several different pathologists the system of grading used may not have been uniform; moreover the cases in which these particulars were given were so few that there were rarely enough in any one site to provide material for an analysis. For all sites combined there were 134 survivors of the 376 patients (35·6 per cent) graded as shown in Table X.

TABLE X.

Grade.	No.	Survived.	Per cent.
I	83	40	48·2
II	153	57	37·3
III	66	17	25·8
IV	74	20	27·0

Table XI shows the 376 cases arranged according to grade and duration of symptoms prior to treatment.

TABLE XI.

Grade.	0–6 months.	6–12 months.	Over 12 months.	Not known.	Totals.
I	36	21	23	3	83
II	74	39	34	6	153
III	40	8	15	3	66
IV	39	22	10	3	74
Totals	189	90	82	15	376
Percentage in Grades I and II	58·2	66·6	69·5	60·0	62·8

TABLE XI—*continued*

Rectum and recto-sigmoid—117 cases.

Grade.	0–6 months.	6–12 months.	Over 12 months.	Not known.	Totals.
I	2	4	2	1	9
II	34	23	19	1	77
III	15	4	3	3	25
IV	2	3	1	–	6
Totals	53	34	25	5	117
Percentage in Grades I and II	68·0	79·4	84·0	40·0	73·5

The differences are not significant. $\chi^2=2\cdot84$, $n=2$, $P<\cdot30>\cdot20$.

Stomach—35 cases.

Grade.	0–6 months.	6–12 months.	Over 12 months.	Not known.	Totals.
I	4	3	3	1	11
II	1	2	1	1	5
III	2	–	2	–	4
IV	9	2	4	–	15
Totals	16	7	10	2	35
Percentage in Grades I and II	31·2	71·4	40·0	100·0	45·7

The differences are not significant, but it has been shown in an earlier section that the highest survival rate among patients with cancer of the stomach was among those in whom the delay in treatment was 6–12 months. $\chi^2=3\cdot47$, $n=2$, $P<\cdot20>\cdot10$.

Scanty as these data are, they are in accord with Bloom's (1950*b*) findings that a higher percentage of patients with tumours of Grades III and IV come early for treatment than of those with the lower grades.

Duration of survival of untreated patients.

Many patients were not treated for various reasons, and the total duration of the disease from first sympton to death is known in most of these. Patients who were in Stage IV of the disease with distant metastases have not been included in the following list, in which the sites are arranged in order of mean duration of survival of patients in Stages I, II and III in whose cases treatment was withheld for other reasons than the advanced stage of the disease (Table XII). The actual duration of survival expressed in months is given, and also expressed as a percentage of the normal expectation of life during the next 5 years for a group of persons having the same sex-age distribution as the group of cancer patients under consideration (Table XII).

The last column of Table XII refers to the three groups into which the sites were divided according to the survival rates in relation to delay in commencing treatment; the figures show that there is no correspondence between these groups and the duration of life in the untreated cases. The mean duration of life in 153 untreated patients in Group A was 23·1 months, of 176 in Group B was 11·7 months and of 296 in Group C was 17·6 months. The sites showing the longest survivals among the untreated patients are those in which treatment by radical surgery alone or with radiotherapy gives the best results, although some sites show a short duration of life, either because, as in the case of the lip, very few cases are left untreated, or because, as in the case of the colon and the intrinsic larynx, untreated patients often die of complications, such as intestinal

TABLE XII.

Site.	Number of patients.	Actual duration in months.	Percentage of normal expectation.	Group.
Breast	53	35·9	72·8	A
Skin, squamous carcinoma	7	34·9	74·6	A
Recto-sigmoid	29	22·4	43·9	C
Bladder, papillary carcinoma	23	22·0	43·3	A
Anus and anal canal	14	21·9	44·5	C
Prostate	43	19·5	39·2	C
Vulva	9	19·1	40·0	C
Rectum, ampulla	132	19·0	38·5	C
Cervix uteri	26	17·5	31·6	A
Corpus uteri	7	17·4	32·9	A
Thyroid gland	8	15·6	29·1	C
Bladder, infiltrating carcinoma	80	14·0	27·0	B
Nasal sinuses	11	13·0	25·2	B
Mouth, floor of	11	12·6	24·7	C
Stomach, pylorus	27	11·5	22·1	B
Mouth, alveolus	5	10·8	22·2	C
,, palate	8	10·0	20·9	C
Lip, upper (1), lower (2)	3	10·0	22·8	C
Ovary, carcinoma	5	9·6	18·0	C
Tongue	14	9·1	17·2	C
Intrinsic larynx	15	9·0	17·9	C
Tonsil	7	8·7	18·5	B
Colon (all sites)	31	8·7	17·4	B
Stomach, mid-gastric	12	8·7	17·6	B
Pharynx	28	8·4	16·3	A
Kidney	9	7·7	13·8	A
Stomach, cardiac	8	5·7	11·3	B

obstruction or broncho-pneumonia comparatively early in the growth of the tumour. In some sites, for example salivary glands, malignant melanoma of skin, penis, testis, sarcoma of soft tissue and of bone, all patients were treated except those whose disease was in Stage IV. The remaining sites in which the duration of survival of untreated patients was less than 12 months—mouth, ovary (carcinoma), tongue, tonsil, stomach and pharynx—are sites in which the results of radical surgery are not so good as in those in which the natural duration of the disease is longer. The conclusion to be drawn is that the growth rate of the tumour is the most important factor influencing the survival rate after radical surgical treatment, leading to relatively long survival in spite of delay in commencing treatment.

SUMMARY.

1. The 5-year survival rates of 2880 patients, whose disease was in Stages I, II and III and who were treated by radical surgery alone or with radiotherapy, were analyzed to ascertain whether the interval between first symptom and commencement of treatment was directly related to the survival rate.

2. It was found that the sites of the disease could be divided into three groups : A. Those in which the highest percentage of 5-year survivals was among the patients treated within the first 6 months from first symptom. B. Those in which it was among the patients treated 6–12 months from first symptom. C. Those in which it was among the patients treated 12 months or more subsequent to the first symptom.

3. Group A included the skin (squamous carcinoma), corpus uteri, penis, cervix uteri, female breast, kidney, bladder (papillary carcinoma) and pharynx. For this group of sites, comprising 1097 cases of known duration, the best survival rate was 51·1 per cent of 616 patients treated in the first 6 months against 42·0 per cent for the remainder. The differences in survival rate were calculated for individual sites, but only those for the skin and one of the differences for the kidney were found to be statistically significant.

4. Group B included salivary glands, tonsil, testis, colon, skin (malignant melanoma), nasal sinuses, bladder (infiltrating carcinoma) and stomach. For this group of sites, comprising 718 cases of known duration, the best survival rate was 30·6 per cent of 134 patients treated in the second 6 months against 27·1 per cent for the remainder. None of the differences in survival rate for individual sites was significant, except that for carcinoma of stomach (all sites) of duration 0–6 months and that for duration 6–12 months, which was 13·0 $\pm$ 6·3 per cent. For all 223 cases of carcinoma of stomach of known duration the survival rates of the cases of 0–6, 6–12 and over 12 months' interval groups tested by the χ^2 test gives the value of $P < \cdot 10 > \cdot 05$ for the difference between observed and expected survivals, which is not statistically significant.

5. Group C included intrinsic larynx, thyroid gland, vulva, lip, bone sarcoma and soft tissue sarcoma, ovary, rectum (ampulla), recto-sigmoid, eye, male breast, anal canal and anus, tongue, mouth and prostate. For this group of 1065 cases of known duration the best survival rate was 53·0 per cent of 266 patients treated more than 12 months from the first symptom against 31·8 per cent of 799 patients treated earlier. In individual sites the only difference which was statistically significant was that between the survival rate of cases of carcinoma of the ampulla of the rectum treated within 12 months of onset, 31·4 per cent and those treated later, 50·8 per cent, difference 19·4 $\pm$ 8·5.

6. The age distribution of the patients was investigated for the most important sites, but in none was any significant difference found between observed and expected survivals due to the differences in age distribution.

7. The highest percentage of patients whose disease was still in Stage I—51·6 per cent—was in Group C, in which the best survival rate was among the patients treated more than 12 months subsequent to the first symptom. It was concluded that the differences in survival rate were probably due to variations in the intrinsic growth rate of the tumours.

8. Only comparatively few observations on the grading of the tumours according to Broders' system were available, but those for carcinoma of the stomach and rectum supported the view that patients with slow-growing tumours formed a high percentage of those who delayed longest before coming for treatment.

9. The total duration of the disease was calculated for 625 patients in Stages I, II and III who were not treated. It was found that the sites showing the longest survivals of untreated patients were those in which the results of radical surgery were most satisfactory.

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THE PROGNOSTIC SIGNIFICANCE OF SMALL SIZE IN BREAST CANCER.

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IT is a strange fact that in the voluminous literature on breast cancer the prognostic value of the size of the tumour is only cursorily dealt with, a fact mentioned by Geschickter (1945) and underlined by Bloom (1950). The explanation probably is, that the view expressed by Kaae (1948), namely, " It is generally recognized that the size of the tumour is a very important factor in the prognosis, and that the latter is much more favourable for the small tumours than for the large," seems so generally sound, even self evident, that the problem has not been considered worthy of a closer study.

If we examine the pertinent literature, we find, however, that the findings are not unequivocal.

Dahl Iversen (1930) states that breast cancers up to the size of plums are without recurrences after 3 years' observation in 83 per cent of the cases, whereas tumours larger, up to the size of a hen's egg, show only 13 per cent freedom from recurrences after 3 years. Eggers, de Cholnoky and Jessup (1941) report upon a 5-year survival of 73 per cent, if the mass is 2 cm. or less, and only 24 per cent if the mass has reached a size of 3 to 6 cm. Haagensen and Stout (1943) found a 5-year clinical cure of 62·2 per cent if the tumour was under 3 cm. and only 19·8 per cent if the tumour had reached 6 cm. or more. They conclude that " The data indicate, as might be expected, that the prognosis becomes worse as the size of the tumour increases ".

Bloom (1950) found 5-year survivals in the following percentages: tumour less than 1 inch 59 per cent, 1 to 2 inches 45 per cent, and more than 2 inches 32 per cent, and he finds after grading of the tumours that: " Of the tumours with a diameter of 1 inch or less, 37 per cent are classified as Grade I and 23 per cent as Grade III. On the other hand, in the case of growths of more than 2 inches diameter only 8 per cent belong to Grade I whilst 54 per cent are Grade III. When the diameter lies between 1 and 2 inches the incidence of these tumours is practically the same ", and he concludes that: " Whether the tumours are small or large, the outlook is uniformly good in the former (Grade I) and bad in the latter group (Grade III). On the other hand, an intermediate result is obtained for the intermediate cases (Grade II), the survival rate being practically halved in the presence of the larger neoplasms. In other words, the metastasizing power for Grade I and also Grade III cancers is independent of size. For growths classified as Grade II this power bears a direct relationship to the diameter, the larger the tumour the greater the likelihood of spread having taken place ". Most probably this holds good also for the Grade I and Grade III tumours, only that

the time factors are so long or so short that their significance is obscured under the usual clinical conditions.

When Kunath (1940) says: "*Size and mass.*—An analysis of this point failed to reveal that a larger tumour carries any more serious a prognosis than does a small one", and Hoopes and McGraw (1942) likewise conclude: "No correlation was found between the post-operative duration of life and the size of the tumour removed", these findings are not necessarily in contradiction to the opposite conclusions, mentioned above. The explanation may be a different composition of the groups of tumours, as regard number of representatives of the different grades of malignancy. Again we have to guard ourselves in transferring conclusions from groups to individual cases.

Considering the importance of assessing the significance of a minimum size of tumour for the prognosis of breast cancer, we have examined breast cancer material consisting of a total of 974 cases. We planned to examine the ultimate development of breast cancers as small as practically diagnosable. In order to decide the upper limit of the size to include, theoretical and practical considerations have been taken into account. Firstly, we considered the smallest lump, distinguishable as a definite tumour. That size evidently depends upon the site and upon the amount of adipose tissue. A tumour near the surface and in a shrunken atrophic breast will be more easily discovered than one deeply situated in a full and developed breast. It seems that tumours smaller than those the size of a pea cannot reasonably be distinguished from the many irregular nodosities in a climacteric, or pre-climacteric breast, and in most cases it is difficult to feel a lump smaller than a small hazel nut. Secondly, we wanted to examine tumours of a size so small that the number of cases would represent a very small fraction of the total, whereby we would obtain a statistical claim to a designation "a very small breast tumour". The size of our tumours is not given in centimetres, or millimetres. The tumours are usually characterized by some object for comparison, usually: pea, bean, nut kernel, hazel nut, date-stone, date, wall nut, plum, etc. A tumour the size of a hazel nut will usually measure some 10 to 12 by 15 to 17 mm. One has to have in mind the small size of a Norwegian hazel nut to appreciate the size.

When we examined our total material we found that only 56 tumours out of 974, i.e., 5·7 per cent. had the size of a hazel nut, or were smaller, and only 10 tumours (1 per cent) were recorded as large as a pea, or a bean. This indicates that we have found a suitable standard, covering our demand for a diagnosis of a breast tumour as small as practically diagnosable. If smaller nodules should be removed for diagnostic or prophylactic purposes, the number of amazons would reach such proportions that a general reaction from the population would be the result.

Our conclusion is further substantiated by the previous papers dealing with this problem. Table I shows the standards and the relative occurrence of the smallest groups recorded:

TABLE I.—*The Maximum Size of the Breast Tumours and the Relative Occurrence of the Size Mentioned.*

	cm.	
Eggers, Cholnoky and Jessup (1941)	1	6 per cent of all.
	2	15 " "
Bloom (1950)	2·5 (1 in.)	50 " "
Haagensen and Stout (1943)	3	15 " "

The present material consists of breasts and lymph nodes received for diagnosis from city and county hospitals, as well as from private surgeons, during the years 1933 to 1942. It is representative, and comparable to any other large, average similar material in Scandinavia. It should comprise cases with at least 75 per cent operability and include a fair number of " early " cases, as the population is rather cancer conscious, being under a constant barrage of cancer propaganda with promises of great hopes of cure if presenting themselves for treatment at an early stage. The fate of the patients has been followed for from 10 to 20 years. The material was examined during the later half of 1952.

The clinical information is not always precise, or detailed, as some of the case histories are very short. Nor is the pathological material always complete, as some of the surgeons do not submit adipose tissue from the axilla if no suspicious glands are found. In other cases a selection of glands only are sent to the laboratory, and finally, some of our paraffin blocks were destroyed during the war, with the result that for some cases a few slides only could be examined. This means that all the positive findings are minimum recordings.

The clinical follow-up has been complete, all patients having been accounted for. Reliable death certificates have been received for all the dead, and the patients alive have been personally examined by one of us, or by competent doctors as regards the patients living in remote parts of the country.

As only the patients with a definite statement as to the *size of the tumour* have been included, the size is known in all cases. The designations have been: slightly larger than a hazel nut, 10 cases; plum stone, 1 case; half a wall nut, 1 case; hazel nut, 22 cases; nut kernel, 11 cases; pea nut, 1 case; pea, or bean, 10 cases.

Grade of malignancy of the primary tumour, 11 cases were recorded as Grade I, 28 cases as Grade II, and 17 cases as Grade III.

Axillary lymph nodes were submitted for examination in 31 cases, lymph nodes not examined in 25 cases.

Treatment varied. In 7 cases a local operation only, and in 7 cases radical operation was performed. In all the other cases the operation was combined with radiological treatment, before, or after the operation, or both. In some cases X-rays were used, in other cases tele-radium. As information is sparse, details are omitted. No conclusions will be drawn as to the methods of treatment, other than to state that the patients have received the varied treatment offered the average breast cancer patient in Norway.

The duration of symptoms, that is, the interval between the first recorded symptom until treatment was commenced, was recorded in 41 cases.

The period of survival has been calculated from the commencement of treatment up to the death of the patient, or the day of clinical examination during 1952.

Results.

In Table II is recorded the findings as regards the question of symptom-free survival for the 5 years' and the 10 years' observation.

Table II.—*Total Number* (*56 Cases*); *Alive, Symptom-free.*

After 5 years	37	66 per cent
After 10 years	36	64 „

In this table all dead during the period are without reservation, counted as dead from breast cancer. No 2 died 14 days after operation, when leaving the hospital. No. 43 died a sudden death 6 months after operation, and No. 8 died from broncho-pneumonia one year and a half after operation, symptom-free. All the others succumbed to their tumours.

This 5-year cure rate corresponds very closely to the rate quoted by Engelstad (1948) for his group of 768 cases, regarded as Stage I and Stage II cases. This indicates that our " small " breast cancers do not present a particularly favourable clinical picture.

In one aspect our Table II shows a remarkable deviation from the usual, namely, the small difference between the 5-year and the 10-year figures, only 1 out of 37 patients dying in the second 5-year interval.

As stated, the whole group of 56 patients has been followed for 10 years. A smaller group has, however, been followed for a longer period, and in Table III some of the results are summarized. The 11 years' observation group ends by the material of the 1941 group, the 12 years' observation material ends by the 1940 group and so on. As therefore the material is not identical in all groups, it is evident that the results may present features which may look inconsistent at the first impression. The findings for the 11th to 14th years are given in Table III.

TABLE III.—*Alive, Symptom-free.*

After 11 years	31	out of	48	65	per cent	(No. 27 died symptom-free. No. 28 and 29 with metastases, dying next year.)
„ 12 „	24	„	40	60	„	(No. 10 died symptom-free. No. 30 with metastases, dying next year.)
„ 13 „	15	„	35	43	„	(No. 31 died symptom-free. No. 20, 24, 32 with metastases, dying next year.)
„ 14 „	8	„	17	47	„	—

Table III shows the very important fact that a considerable number of patients arrive at the 10 years' limit in apparently good condition, but that nevertheless they are bearers of silent metastases, which eventually terminate their lives.

As 17 patients only, out of the total of 56, have been followed for as long as 14 years, a number of fatal metastases are probably due among the remaining, and we may conclude that more than half of all the patients presenting themselves with a tumour, the size of a small hazel nut, or smaller, are potential victims of

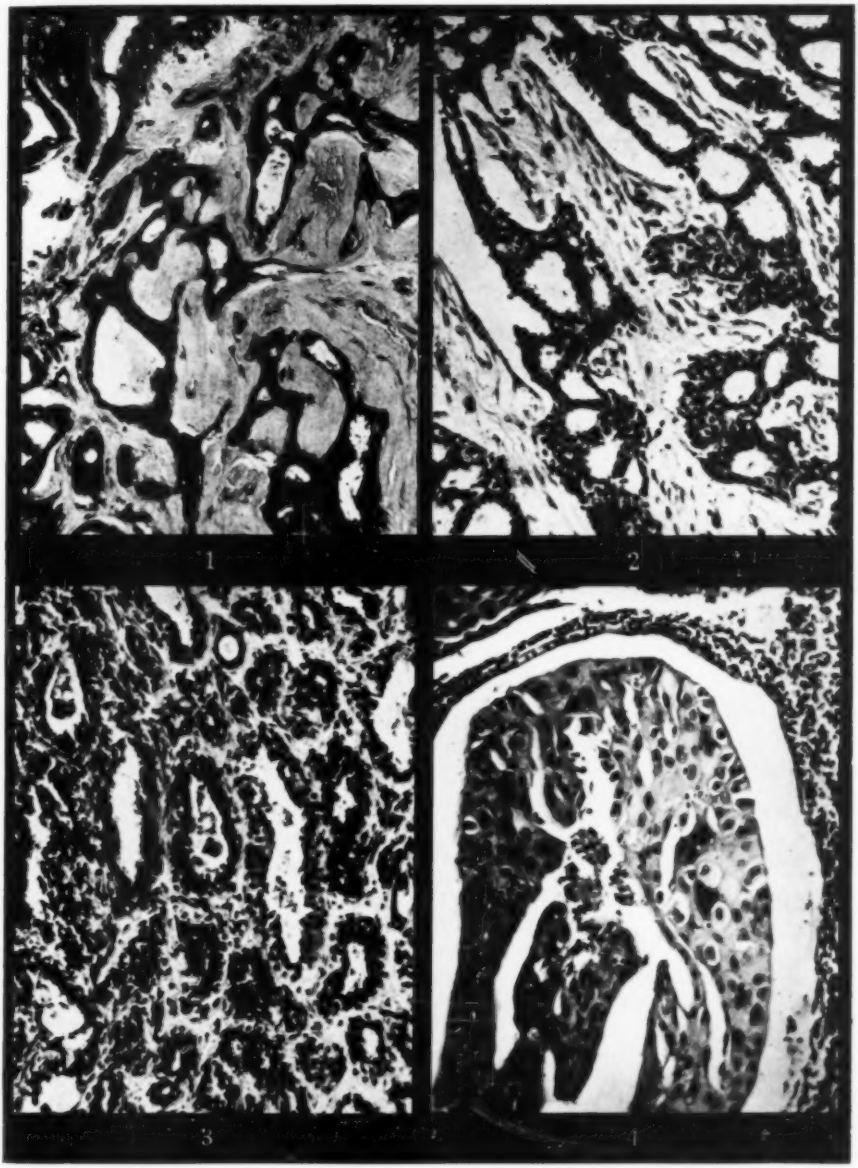
EXPLANATION OF PLATE.

FIG. 1.—G. N—, 61 years old. J. No. 280/38. Carcinoma Grade I. Size: hazel nut. Symptoms two weeks. Radical operation. Post-operative radium treatment. 14 years' observation. Symptom-free. × 135.

FIG. 2.—W—, 48 years old. J. No. 2507/40. Carcinoma Grade I. Size: hazel nut. " Brief " case history. Radical operation. Post-operative X-rays. Symptom-free, 12 years' observation. × 135.

FIG. 3.—M. E—, 55 years old. J. No. 1721/40. Carcinoma Grade I. Size: bean. Unknown duration of symptoms. Radical operation. Post-operative X-rays. Symptom free, 12 years' observation. × 135.

FIG. 4.—L. S—, 60 years old. J. No. 554/40. Carcinoma Grade III. Size: hazel nut. Symptoms a few days. Radical operation. Three lymph nodes and perinodular adipose tissue infiltrated by tumour cells. The picture is from one of the lymph nodes. Post-operative X-rays. Symptom-free, 12¾ years' observation. × 155.



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their cancer, when given the average treatment in use in Norway. Even if we select the very smallest tumours present in our series, those the size of a pea or a bean, out of a total of 10, we find only 5 patients alive after 10 years, or more, and 4 patients dead from metastases.

In the present series the combined surgical and radiological treatment seems, in a number of cases, to have effected a considerable delay in a finally fatal outcome. This delay is of very great value from a therapeutic standpoint, even if the treatment fails to effect a complete cure.

The prolonged observation also helps to assess more accurately the real stage of the tumours. Staging actually is an ambiguous process. The simplest and least reliable staging is the immediate clinical, unreliable to a degree to make it practically useless. The anatomical staging is somewhat more precise, and, in lack of anything better, the accepted basis for grouping of tumours for comparison of therapeutic results. We have, however, to bear in mind that the value even of this grouping is rather limited. Negative findings of tumour cells in lymph nodes removed may be the result of the pathologist not finding the cells in spite of their presence. Or, tumour cells may by-pass the usual lymphatic filters in the axillary glands and proceed directly to the supraclavicular or the intrathoracic nodes, or the tumour cells may spread via the blood stream. The discrepancy between the number of tumours anatomically staged as Stage I and the number of cures effected after a proper local operation shows the degree of failure in the process of staging. The average failure is generally at least 20 per cent. The final and most accurate staging is a retrospective process, taking into consideration the outcome of the cancerous growth. A number of patients staged as Stage I eventually through the clinical development are shown to be in Stage II, or further. On the other hand, if radiological treatment has been added to the surgical, even the delayed retrospective clinical staging may be incomplete, because a tumour, anatomically staged as Stage I and actually being in Stage II, may be cured by radiological treatment and thereby escape its proper staging.

In spite of the incomplete clinical and pathological information, it will be of some interest to examine the state of affairs, as regards stages recorded in this material. The data available are shown in Table IV.

TABLE IV.—*Stages : Status by the end of* 1952.

	Alive, symptom-free. Minimum 10 years.		Dead, and patients living with metastases.	
Lymph nodes negative	13		7	
One lymph node invaded	3	5	1	6
Several lymph nodes invaded	2		5	
State of lymph nodes unknown	9		16*	
Total	27		29	

* No. 10, 27, 31 died symptom-free after more than 10 years' observation.

The large number of fatal outcomes with negative findings of tumour cells in the axillary lymph nodes confirm our assumption that our figures represent minimum findings. The considerable number of patients alive and symptom-free in spite of positive findings of tumour cells in the lymph nodes, actually 5 patients, further confirm our statement as to a considerable effect of the treatment, but

also underlines the great number of patients already in Stage II (or further) at the moment of presenting a very small breast cancer.

If we add these 5 patients with microscopical lymph-node metastases, but still symptom-free, to the group of the dead and those living with metastases, we find that two-thirds of all our patients with small tumours were in Stage II (or further) when the operation was performed.

The value of Bloom's (1950) grading and analysis of his material made it natural to try his principles on our material. We deliberately use the word principles, because any grading is arbitrary, and the designation in each case is the result of a subjective estimate of a series of characteristics, which themselves are unprecise. With this reservation, which possibly will be further substantiated by our findings, we present our material in Table V.

TABLE V.—*Grades : Status of our Material by the end of* 1952.

	Alive, symptom-free. Minimum 10 years.		Dead, or patient living with metastases (known).	
Grade I	11		0	
„ II	8	15	20*	30
„ III	7		10	
Total	26		30	

* No. 10, 27, 31 died symptom free after more than 10 years' observation.

This survey confirms the importance of recognizing and considering the Grade I cases when statistics are prepared of breast cancer materials. All our Grade I patients, a total of 11, and 20 per cent of the whole group, are alive, one of them having presented tumour cells in an axillary lymph node. It may be pertinent to note that all these tumours designated as Grade I are histologically definite carcinomas, not merely cases of " atypical proliferation ", " carcinoma *in situ* ", or similar conditions (Fig. 1, 2, 3). On the other hand, however, we have not succeeded in registering clinical evidence of an increasing malignancy with the passing from Grade II to Grade III. The explanation may be that the number of cases is too small, that the treatment given may have influenced the final result because a number of very malignant tumours are comparatively radiosensitive, or it may be caused by our incompetency. We reproduce the histological picture of one of our most malignant tumours (Case No. 36), recorded as Grade III and with 3 lymph nodes invaded. The patient is still alive and feeling well nearly 13 years after the diagnosis was made (Fig. 4). She had radical mastectomy, and afterwards was given a series of X-ray treatments at one of our county hospitals (Telemark Fylkessykehus). This case, as well as some others, show that in individual cases all hope shall not be given up, even if the situation looks very dark.

If we return to our Grade I cases, we find that these tumours represent a little more than 40 per cent of all the patients alive and symptom-free. If we therefore deduct these tumours from the material assembled in Tables II and III we find a considerably less favourable result, as shown in Table VI.

Table VI shows that if we exclude the Grade I tumours, which actually have a very low malignancy, approximately two-thirds of the patients in our series have died or will die from their breast cancers.

TABLE VI.—*Alive and Symptom-free. All Cases, less Grade I.*

Years.		Per cent.
5	26 out of 45	58
10	25 „ 45	56
11	20 „ 37	54
12	14 „ 30	47
13	8 „ 28	29
14	5 „ 14	36

In Table VII are collected the data as regards duration of symptoms and prognosis.

TABLE VII.—*Duration of Symptoms and Prognosis.*

	4 weeks.	2–3 months.	4–6 months.	7–12 months.	More than 1 year.
Alive, symptom-free	10 (4)	4	2 (2)	1	5 (3)
Dead, or alive with metastases	6	3	2	6	2

This table does not include all cases, but only those where the duration of symptoms before treatment is known. Grade I cases in brackets.

At a first sight there seem to be two comparatively favourable periods: a relatively good prognosis for the patients with a very short (4 weeks) and for those with a very long (more than a year) history of symptoms. The medium long histories, from a few months up to a year, show an increasingly gloomy picture.

If we again deduct the Grade I cases we conserve the pattern, and still find indications of a benefit for very quick reaction with immediate treatment after diagnosis, and with a still more pronounced bad prognosis in cases with delays from a few months up to a year. For the very long histories (more than a year) the comparatively better prognosis is preserved, and may partly be accounted for by a default in the process of grading, partly by combination of high malignancy and high grade radiosensitivity in certain cases, and partly by inclusion of tumours with a more moderate malignancy (Grade II). Besides, a perfect correspondence between morphological grading and biological development is evidently not obtainable. Too many unknown factors are involved here. To elucidate this question a larger material is necessary.

SUMMARY AND CONCLUSIONS.

The present study of the fate of patients presenting mammary carcinomas the size of a hazel nut, or smaller, shows that one half of the group have eventually succumbed to their tumour. If we deduct the Grade I tumours, one-third only of the patients survives. At the commencement of treatment two-thirds of the patients are in Stage II, or further.

The combined surgical and radiological treatment seems to have effected a considerable delay in the progress of the cancerous growth, and in some cases to have resulted in a complete cure, even when axillary metastases were present. The delayed development of the tumours is manifested by an insignificant difference in the 5 years (66 per cent) and the 10 years (64 per cent) figures for "alive symptom-free" and by the occurrence of a significant number of metastases after the tenth year of observation after treatment.

Conclusion.—The small malignant mammary tumours give, as a group, not decidedly, or appreciably, more favourable prognosis than an average group of breast cancers belonging to the ordinary average group of Stage I and Stage II cases undergoing the same type of treatment.

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SKIN CANCER IN THE SOUTH AFRICAN BANTU.

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SKIN cancer is relatively infrequent in the African. In the three-year period 1949 to 1951, during which time 590 cases of malignant disease in Africans were referred to the Radiation Therapy Department, only 50 cases of skin cancer were encountered. By comparison, among 1642 cases of cancer in Europeans seen during a similar period, 540 primarily affected the skin. It is, of course, realised that in the relatively primitive African community only a small fraction, generally estimated at about one-quarter of affected persons, attend hospital for treatment. However, this does not alter the fact that skin cancer accounts for only 8·4 per cent of our series of malignant disease seen in the African, compared with 33 per cent in the European. Relatively low incidences of skin cancer in pigmented races have been reported from other sources. In a series of pathological specimens originating from natives of Nigeria, Smith amd Elmes (1934) showed that primary skin cancer (including melanoma) accounted for 15 per cent of all tumours received. Schrek (1944), found that skin cancer accounted for only 3 per cent of tumours in American Negroes (male military personnel), compared to 19 per cent among white persons in a similar category. Confirmatory data for other groups of pigmented people have been obtained by Khanolkar (1950, 1951) in India, by Kouwenaar and Sutomo (1951) in Indonesia, and by Mussini-Montpellier (1951) in French North Africa. In addition skin cancer in pigmented races differs from that commonly seen in white skinned persons in many other respects, apparently occurring on a different basis with a distinct anatomical distribution generally appearing on the lower extremity, and often at sites of long-standing chronic infections or post-traumatic scars.

In a previous publication (Cohen, Shapiro, Keen and Henning, 1952) we showed that skin cancer in European residents in the Transvaal appeared almost exclusively on the face and hands (98 per cent of cases), and was exceedingly frequent in fair-skinned persons engaged in outdoor occupations. It was suggested that these observations could best be explained by the carcinogenic action of the atmospheric ultra-violet radiation, which, in the climatic conditions of the South African high veld, reaches an intensity among the highest in the world (Richards, 1939; Riemerschmid, 1940). The pigmented skin of the African would, of course, be largely protected from this effect, and indeed when skin cancer does make its appearance, it is almost invariably in areas exposed, not to sunlight, but to other types of chronic irritation.

We have also observed distinct differences in pathogenesis, and the rate of growth and dissemination of skin tumours in the African, as compared with the

classical description of the disease in Europeans. These factors have compelled us to adopt a different approach to the management of skin tumours in the two races, and to revise our views on the surgical and radiological curability of skin cancers of various histopathological types. Since these racial differences might be of some interest in the study of the demography, endemiology, and management of cancer in Africa, it was considered worth while putting our experiences on record at this stage.

The population from which this material is drawn consists of approximately 2,000,000 Africans of the Bantu race inhabiting the southern half of the Transvaal, and originating for the most part from the Sotho and Zulu tribes. About one-quarter of this population is urbanised and resident on the Witwatersrand, but a much larger fraction of the hospital population gives an urban address. This is due, in part, to many rural patients living with relatives in the urban area prior to entering the hospital and witholding their true addresses, and also to the greater tendency among rural natives to receive treatment from indigenous herbalists rather than in hospitals. In the absence of suitable vital statistics it is impossible to state the age distribution of the Bantu population, although it is safe to assume that the average age is somewhat lower than that of the European group. The ratio of males to females at the time of the 1946 census was 1·33, the predominance of males presumably reflecting the migratory labouring class.

From the genetic point of view it is important to bear in mind that the Bantu inhabitants of South Africa originate from Central and East African tribes, who have, at least within historic memory, had no admixture of European blood. By comparison, the Negro of the United States originates from West African ancestry and is largely a product of prolonged hybridisation. Although environmental conditions in the southern states of the U.S.A. are not dissimilar to those encountered by the Bantu in the Transvaal, the two groups generally show a very different incidence and distribution of malignant disease (Higginson, 1951 ; and Robinson, 1951). With the exception of melanoma, however, the incidence and distribution of skin cancer in the American Negro as described by Schrek (1944) is quite similar to that in our series, suggesting that the genetic constitution, apart from pigmentation, is a relatively unimportant aspect of the disease. That the low incidence of skin cancer in the African cannot be explained on the basis of a racial immunity is clearly shown by the very high incidence of both squamous and basal cell carcinomas in the African albino, who accounted for 12 of our series of 50 cases, and in whom the anatomical distribution of the lesions was identical to that observed among the Europeans. It appears, therefore, that pigmentation by virtue of its protective action against sunlight is the most significant factor in determining the incidence and distribution of skin cancer.

The histopathological types of malignant skin tumours encountered in both pigmented and albino Bantu during the period under review are shown in Table I.

TABLE I.—*Cases Grouped according to Histological Diagnosis.*

Histopathology.	Pigmented Bantu.	Albinos.	Total.
Squamous cell carcinoma	25	9	34
Basal cell and sebaceous carcinoma	3	3	6
Malignant melanoma	10	0	10
Total	38	12	50

It will be noted that metastatic tumours of the skin arising from primary cancers in other sites have been excluded from this series, and that melanoma is here classified as a primary skin cancer. We have not considered the so-called haemangiosarcoma of Kaposi as a skin neoplasm, although it is a relatively common feature in the Bantu, accounting for almost 40 per cent of all cutaneous tumours seen in our clinic.

Squamous Cell Cancer.

During the period under review we have seen 25 cases of squamous cell cancer in the pigmented Bantu. The distribution of the lesions and their apparent aetiology is of interest, since they differ so greatly from the squamous carcinomas encountered in the white skinned population.

Sex distribution.

Seventeen cases occurred in males and 8 in females, a ratio similar to that encountered in Europeans.

Anatomical distribution.

The sites involved in squamous carcinoma of the Bantu are shown in Table II, together with comparative data in Europeans (after Cohen *et. al.*, 1952).

TABLE II.—*Anatomical Distribution of Squamous Cell Carcinoma.*

	Bantu.		European.
	Cases.	%	%
Head and neck	10	40	89
Lower extremity	11	44	1
Trunk	3	12	1
Upper limb	1	4	9

It is of great interest to note the relatively high incidence of these tumours on the trunk and lower limbs in the pigmented population as compared with white skinned people.

Aetiology.

In every case, where an adequate history was obtainable, a definite aetiological factor was established as the basis for the development of the malignant skin tumour. These factors undoubtedly play a large part in the unusual anatomical distribution of the lesions.

One or other of the following factors was present in this series of cases :

(i) *A syphilitic basis.*—Syphilitic scars were shown to have been present at the site of the tumour in 3 cases.

(ii) *Old burns.*—At least five of the epitheliomas arose in the scars of old burns, the original trauma having occured from 10 to 25 years prior to the development of the tumours (Fig. 1).

(iii) *Other trauma.*—Injury of some kind was definitely shown to have been present in 2 cases.

(iv) *Tropical ulcer.*—In one case an antecedent tropical ulcer had been the basis for the development of a squamous cell skin cancer (Fig. 2).

(v) *Keloid.*—In 2 cases the tumours had developed in pre-existing keloids.

The above findings show that the skin pigmentation does act as a protective mechanism against ultra-violet radiation in exposed parts, but that under the stress and strain of chronic irritative pathological processes any portion of the body may be affected. There is thus no absolute racial immunity to the development of skin cancer in the Bantu, although the pigmented members of the population are considerably less prone to the development of skin cancer than the white skinned population. It is of interest to note that the clinical picture and the therapeutic problem of skin carcinoma in the Bantu is almost identical with those rare cases of skin neoplasms occurring in chronic varicose ulcers or chronic eczemas described in the European (Black, 1952).

Age incidence.

The average age of the cases in this series was 59·4 years, the youngest patient being 17 and the oldest was 75 years old. It should, however, be stated that often the patient does not know his age, and on these occasions a rough estimate is made, so that the figures given here must be accepted with some reserve.

Stage of disease.

Analysis of the extent of the disease showed that in only 4 cases could the disease be classified as early (Stages I and II), and the remaining 21 cases showing advanced tumours (Stages III and IV) with enormous primary lesions, massive regional lymph node involvement, and often local bone invasion.

In contradistinction, in the white skinned patients 97 per cent had early lesions, and only 3 per cent showed advanced disease.

Treatment.

Owing to the bizarre nature of these tumours, their advanced stage, and the usually unhealthy condition of the surrounding tissues or "tumour bed", the management of these cases was seldom simple.

EXPLANATION OF PLATES.

FIG. 1.—Squamous carcinoma of the foot arising at the site of a burn received many years previously. The tumour invaded bone, necessitating amputation.

FIG. 2.—Squamous carcinoma of the lower leg arising in a pre-existing tropical ulcer of long duration. This case was treated with a radium mould, followed by plastic surgery.

FIG. 3.—Sebaceous gland carcinoma of the scalp (Case 2).

FIG. 4.—Microscopic section of tumour from Case 2. "The histological features are those of a carcinoma of sebaceous glands." Edge of focus of basal cell tumour mass showing gradual transition to lipoid-containing sebaceous cells. 16443 F. H. & E. × 90.

FIG. 5.—Case 2, eleven months after irradiation with 5100 r delivered in five weeks.

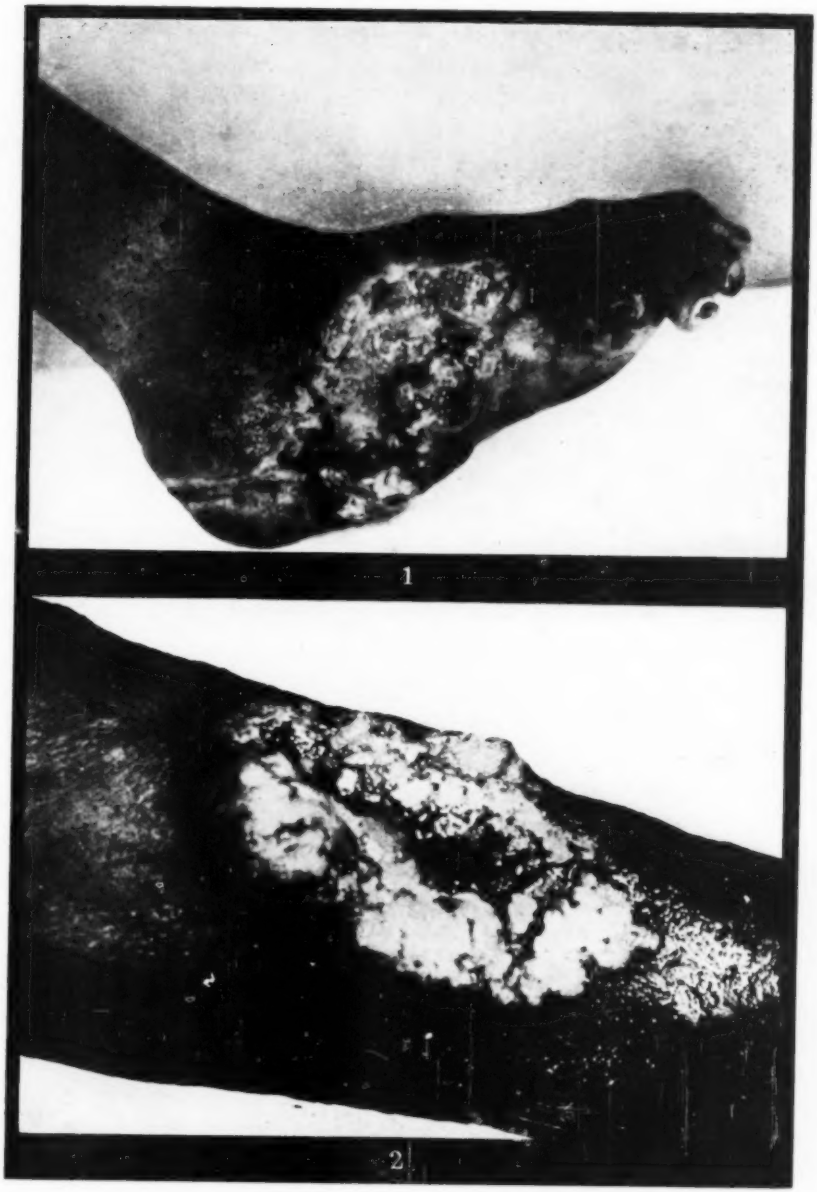
FIG. 6.—Squamous epithelioma in the right supraclavicular region in an albino, also showing multiple hyperkeratoses of the face and chest.

FIG. 7.—Microscopic section of a typical melanoma of the sole of the foot in an adult Bantu male. Malignant melanoma before therapy. 8353 E. H. & E. × 85.

FIG. 8.—Section from the same tumour (Fig. 7), excised a month after receiving 6000 r in 25 days, showing persistent, apparently viable cells. Malignant melanoma after therapy. 10776 E. H. & E. × 130.

FIG. 9.—Typical melanoma of the foot occurring in an elderly Bantu female.

FIG. 10.—Same patient as Fig. 9, 3 months after irradiation with 6000 r in 4 weeks, showing no macroscopic tumour.



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Usually either surgery alone or radiotherapy alone would have been doomed to failure, because of the extent to which the disease had progressed when the patients were first seen at hospital, and therefore both methods were required. Many cases had their initial treatment carried out at an outside hospital, but ideally these patients would probably have benefited from combined surgical and radiotherapeutic procedures.

Surgery as the sole method of treatment was carried out in only 3 cases, and was in each instance an amputation of a limb combined with block dissection of the regional lymphatic glands ; surgery and radiation as a combined treatment was instituted in 10 cases ; radiation alone was used in 11 cases, and palliative treatment only was carried out in one case.

Radiotherapy.—The radiotherapeutic techniques employed were varied, since in most cases the " therapeutic ratio " (the ratio between the tolerance of the normal tissues and the tumour curative or lethal dose) was low because of the large areas involved by these massive tumours. These large areas had to be subdivided, and strip field techniques, using strips about 3 cm. wide, were employed in some cases. In others " grids" were used after Jolles' (1949) method. These procedures allowed much larger dosages to be delivered to the tumours than could be given by the more conventional large field X-ray therapy techniques. By so doing the therapeutic ratios were increased, and in many cases the immediate tumour responses were very satisfactory.

In only 2 cases was medium voltage (135 kv.) X-ray therapy used, the remaining cases being treated with X-rays generated at 200 to 250 kv. In 3 cases interstitial radium was used in addition to X-ray therapy. Surface radium moulds were used in 3 cases in which the tumours occurred on the lower limbs (Fig. 2).

Basal Cell Cancer.

These lesions are extremely rare in the Bantu and we have only one case in our series. This occurred in an adult male who was referred to hospital after incomplete excision of an ulcer of the back which was histologically shown to be a basal cell carcinoma of hair matrix origin. This was treated by means of medium voltage (135 kv.) X-rays, and the patient is quite well and free from disease 2½ years after treatment. The area was treated by means of a 5 cm. diameter circular application at 16·5 cm. F.S.D. to a dose of 4000 r in 5 days using 2 mm. Al filter.

Two Unusual Scalp Tumours and a Sebaceous Gland Carcinoma.

These cases were of interest and will therefore be given separate and detailed description.

Case 1.

Female aged 38. History of 7 years' swelling over occiput which had gradually increased in size. On examination there was a large ulcerated tumour over the occiput measuring 12 cm. × 11 cm. and raised up 10 cm. from the surrounding scalp surface. There were no palpable cervical lymph nodes.

Histological report.—" Section of this specimen from the scalp shows the histological features of a well differentiated, low-grade squamous carcinoma ".

Treatment.—This patient was treated by means of X-rays generated at 200

kv. with 0·5 mm. Cu. filter, H.V.L. = 1·0 mm. Cu., 50 cm. F.S.D., using 2 lateral opposing fields to the occipital region. Dosage was 4200 r to each field fractionated over 4 weeks. The estimated minimum tumour dose was 5000 r.

When last seen there was some residual tumour present, but the patient refused further treatment and up to the present time she has not been seen again.

Case 2.

Male aged 68. Admitted to hospital having had a tumour in the occipital region of the scalp for many years, it being impossible to obtain from the patient the exact length of time that the tumour had been present. A large fungating tumour of the scalp over the occiput was present measuring 12·5 cm. × 10 cm., and raised up 7·5 cm. from the surrounding scalp surface (Fig. 3). There were no palpable cervical lymph nodes present. X-ray of the skull revealed some areas of destruction in the underlying bone.

Histological report.—" Section of this specimen from the scalp shows the presence of a malignant epithelial tumour. The cells are arranged in the form of distorted, branching tubules or of alveolar masses. In some areas the cells are separated from the surrounding stroma by a well-defined basement membrane while in other areas the cells appear to infiltrate into the stroma. The basal layer of cells is composed of fairly uniform polygonal cells with a faint basophilic cytoplasm and vesicular nucleus. As the cells approach the lumen they become larger, the cytoplasm becomes faintly eosinophilic and sometimes vacuolated. The nucleus becomes smaller, more dense, and shows a well-defined nuclear membrane. The nucleus in these cells lies centrally, and their whole appearance is that of lipoid containing cells resembling those of the sebaceous glands. Some of the cystically dilated glands are filled with necrotic debris and disintegrated cells. The tumour is covered by acute inflammatory cells.

" The histological features are those of a carcinoma of sebaceous glands " (Fig. 4).

Treatment.—X-ray therapy, 200 kv., 0·5 mm. Cu. filter, H.V.L. = 1·0 mm. Cu., F.S.D. = 50 cm., was delivered through two lateral opposed fields to the occiput, a minimum tumour dose of 5100 r being given in an overall treatment time of 5 weeks. After 6 weeks there was a dramatic response to the treatment and at the present time, 11 months after treatment, the affected area is completely healed with no sign of any active tumour (Fig. 5).

One other case of sebaceous gland carcinoma came under our care.

Case 3.

This patient was a male aged 39. He was admitted from an outside hospital with a history of having had a tumour of the right upper arm excised 10 months previously, and that the lesion has subsequently recurred. There was a massive tumour present on the right upper arm and shoulder region, surrounding a vertical scar of the old excision. No palpable lymph nodes were present.

Histological report.—" Section of this specimen from the shoulder shows the presence of fibrous connective tissue and a mass of malignant epithelial cells covered by hyperplastic papillomatous squamous epithelium. In some areas the tumour cells show an alveolar arrangement, but the greater part consists of a solid mass of cells. The majority of these cells are round or polygonal, of mod-

erate size, with round or oval nuclei, distinct nucleoli and a fairly thick reticular chromatin network. The cells contain a slightly basophilic, vacuolated cytoplasm with an indistinct cell membrane so that in one or two areas sheets of cells appear to form a syncytium. In the superficial part of the section the tumour cells are separated from one another by oedema fluid.

"The histological features are those of spheroidal cell carcinoma of sebaceous gland origin".

Treatment.—The patient was treated by means of X-ray therapy, 200 kv., 0·5 mm. Cu. filter, H.V.L. =1·0 mm. Cu., 50 cm. F.S.D. Owing to a technical oversight a dose of only 2000 r was given to both an anterior and a posterior field applied to the affected area in the overall peroid of 12 days. There was marked tumour regression after this treatment, and the patient was allowed to return home. Three months later he was readmitted to hospital with a massive recurrence in the treated arm. He was given further X-ray therapy, and a dose of 4000 r was delivered to anterior and posterior fields over a period of 4 weeks. Again there was an excellent response to treatment, but within a few weeks he developed severe pain in the right upper arm, and X-rays revealed extensive bony destruction of the humerus. Three months later he had a disarticulation at the shoulder joint in order to relieve the intense pain. He then developed definite tumour on the anterior chest wall and at the wound edges. He was given further palliative X-ray therapy, but 2 months later developed metastases in the 3rd and 4th lumbar vertebrae. He became very ill, and was transferred home after further palliative X-ray therapy to the lumbar spine.

The 2 cases of "sebaceous gland carcinoma", although showing advanced disease, clearly illustrate the extreme radiosensitivity of these tumours, with conventional large field X-ray therapy. One patient is free from disease, while the second has extensive and incurable tumour.

Albinism and Cancer of the Skin.

It is remarkable that there were 12 albinos among the 50 cases of skin cancer reviewed, although the albino trait is rare in the African. The exact incidence of albinism in the Bantu is not known, but a cursory review of recent hospital records suggests that it cannot be greater than 1 in 5000, and is possible less than 1 in 10,000. Consequently the 12 patients seen, representing the appearance of 4 new new cases annually (and probably several more who do not attend hospital), implies that a large proportion, if not all of the albinos among the Transvaal Bantu necessarily develop skin cancer in early adult life. This observation is in agreement with the experimental findings of Findlay (1928), Beard, Boggess and von Haam, (1936) and the recent precise quantitative investigations of Blum (1950), who have demonstrated that albino animals exposed to daily insolation or ultraviolet irradiation for a significant fraction of their life-spans invariably develop malignant skin tumours.

All 12 patients showed complete albinism of the whole skin surface including the hair and eyes. We did not encounter any cases of partial albinism, such as those described by Gelfand (1944) and shown to have the same propensity for skin cancer as the true albino. However, there was one case of *Xeroderma pigmentosum* (not included in Table I) occurring in a 4-year-old Bantu child in whom the whole skin surface showed the characteristic regular mottling caused by depigmented patches alternating with areas of increased pigmentation. The

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pigment-free zones, especially those of the head and neck, possessed the characteristic hypersensitivity to light and were the origin of several histologically proved squamous carcinomas.

There were 8 males and 4 females in the series. The age distribution of skin cancer in albinos shows a distinct tendency for the disease to occur in younger age-groups than in normally pigmented persons. There was 1 patient in the second decade, 3 in the third, 5 in the fourth and 3 in the fifth decade. The average age was 35 years (compared to 59 years in the pigmented group).

The histological diagnosis showed typical squamous cell cancer in 9 cases and basal cell tumours in 3 patients. One of the basal cell carcinomas was a rodent ulcer similar to that usually encountered in Europeans, while the remaining two were clinically characterised as hyperkeratosis. In all of the 9 cases classified as squamous cell cancer the diagnosis was based on examination of the main presenting lesion, although most patients had several other early keratotic lesions, many of which showed predominantly basal cell proliferation.

The anatomical distribution of the tumours was similar to that observed in the European, in that they tended to localise on exposed areas, and bore no resemblance to the distribution characteristic of the pigmented Bantu. In 10 cases the head and neck were primarily involved, and in 2 the lesions first appeared in the shoulder region (Fig. 6). Apart from the major lesion, every case had innumerable keratoses dispersed over all exposed areas, including the face, neck and shoulders, both hands and forearms, and the lower legs and dorsa of both feet. This distribution should be viewed with cognisance of the scanty clothing worn by rural Bantu during childhood and adolescence. Since the abdomen and thighs are the only areas traditionally clothed, it is not surprising that these areas alone are consistently free from the pre-cancerous dermatosis.

The staging of the disease was determined largely by the multiplicity of the lesions rather than size or extent. Eight patients could be categorised as " early " cases, but since each required treatment to more than one lesion, they are classified as Stage II. The remaining 4 cases had glandular metastases, 2 with underlying bone erosion, and were consequently placed in Stages III and IV respectively.

Treatment in Stage II cases consisted of radical irradiation of each established lesion with the standard therapeutic techniques, followed by prophylactic measures directed against the residual hyperkeratoses. Radiation has not been found practical for the eradication of extensive keratoses on account of the large skin areas involved, but the use of podophyllin, as suggested by Sullivan (1949) and Larsson (1950) for the treatment of early rodent ulcers, would seem to offer a satisfactory solution to this problem. We have found that an ointment of 20 per cent podophyllin in a water-soluble base applied to each keratotic patch twice daily for 8 to 10 successive days is quite adequate for this purpose. Where large areas are involved, the ointment has given immediate results superior to irradiation, leaving after a transient desquamative reaction, apparently normal skin, with none of the atrophy, scarring, or telangiectasis associated with curative doses of radiation. In the advanced cases a combination of surgery and radiotherapy was employed in the hope of eradicating the disease, although the prospect of cure was considered remote.

Since the albino skin has an unusually great propensity for the development of cancer, and is almost invariably the seat of a multiplicity of recognisable precancerous stigmata early in adult life, energetic prophylactic measures are required

in order to avoid, as far as possible, induction of the tumour in the first instance and to prevent the appearance of further primary tumours following treatment of established lesions. Avoidance of direct exposure to sunlight is obviously desirable, and in our experience some form of sheltered indoor employment is the only practical remedy, especially as the albino invariably suffers from photophobia, nystagmus, or some more serious visual defect. It is possible that a suitable protective ointment as suggested by Smithers and Wood (1952) for the control of *Xeroderma pigmentosum* might be of value.

On the appearance of obvious keratosis, treatment with the podophyllin ointment described above is probably efficacious. We have not had the opportunity of observing the subsequent history of skin areas cleared of all macroscopic keratoses by podophyllin, and, in consequence, are not able to assert that the onset of skin cancer is, in fact, prevented. However, Berenblum's (1951) observations on the definite anticarcinogenic effect of podophyllin, even in the presence of actively carcinogenic hydrocarbons, suggests that this material may have a true prophylactic action. We have thus far not observed recurrence of keratoses or the onset of carcinoma within skin areas so treated.

Malignant Melanoma.

A considerable amount of literature has accumulated during the last 15 years on the subject of malignant melanoma. Most authors are agreed that this is one of the most malignant tumours in human beings, and that radical surgery in the early stages gives the only hope of cure, though Ellis (1939) has suggested that sufficiently intensive irradiation might prove useful, and Hergarten and Hergarten (1951) have shown that results after radiotherapy in early cases were better than with radical surgery.

All statistics from the United States of America have shown that the malignant melanoma is relatively rare in the American Negro, though Enos and Holmes (1951) have suggested that it is more common in the Negro in the tropics, and runs a less virulent course.

Our experience with the South African Bantu has shown certain discrepancies with the generally accepted views, and they are, in our opinion, of sufficient interest to warrant publication.

Frequency.

As stated earlier, skin cancer as a whole is relatively rare in the Bantu and represented only 8·4 per cent of the total malignancies in this series, whereas in Europeans it represented 33 per cent of cases. However, out of the total of 50 Bantu skin cases, 10 were melanomas, which is a very high proportion when compared to similar series in America (Muelling and Burdette, 1950).

According to the Annual Reports of the South African Institute for Medical Research, over a three-year period, during which over 4000 malignant structures were examined, malignant melanomas accounted for 2·29 per cent of cases in Europeans as compared to 3·38 per cent in the Bantu. These figures suggest that the malignant melanoma is at least as frequent in the South African Negro as in the European, which is contrary to the findings in the two races in the United States of America.

It is of interest to note that despite the relatively large numbers of albinos in this series there was not a single case of malignant melanoma in an albino. During the last 10 years at the Non-European Hospital, Johannesburg, no malignant melanoma has been found in an albino.

Clinical features.

In all cases in this series the local lesion was in an advanced stage, and some were kept under observation for sufficiently long periods to enable the clinical progress of the disease to be observed.

In this series, and during the last 10 years at the Non-European Hospital, not a single case of distant metastasis has been noted, all cases having remained regionally localised to the lymphatic system, but the spread is more rapid than for the average carcinoma.

This clinical progress can best be illustrated by a case which was kept under observation till termination.

Case 4.

Female, aged about 45, admitted with the following history: Three months before admission she had noticed a black pimple on the lower portion of the right thenar eminence which grew rapidly—a few weeks later she developed a lump in the axilla and subsequently the whole arm swelled up. Two to 3 weeks before admission black lumps appeared on the skin in the elbow region.

On admission the patient had a typical malignant melanoma over the thenar eminence of the right hand. The arm, forearm and hand were oedematous.

There was a mass of hard matted glands, 10 × 8 cm., in the axilla, and numerous melanotic nodules on the flexor aspects of the forearm and arm, mainly in the elbow region. The patient's general condition was good, and there was not much disability, and relatively little pain.

The primary growth was irradiated as well as numerous smaller areas with varying dosages and time factors, and biopsies obtained before and after treatment. 200 kv. X-rays were used with 1 mm. Cu and 1 mm. Al filtration, giving a half value layer of 1·5 mm. Cu. One area was treated with the "chessboard" technique with a single dose of 3000 r to each set of squares. The primary growth and axillary glands were given 250 r daily to a total of 5500 r, other areas were treated with 1500 r and 1000 r daily to totals of 4000 r, 4500 r, and 6000 r, and six small areas were treated with three daily doses of 2000 r. All treated areas responded clinically by regression of the tumours and some necrosis of the tumour surface, but histological examination after the radiation showed minimal changes, A few lesions treated with 1000 r daily for 6 days disappeared completely. Six weeks after admission it was noted that the supraclavicular glands were enlarged and painful, but the intervening skin appeared normal. Ten days later small black nodules were visible in this skin area. The next extension was to the areola of the right breast, which became indurated, more pigmented and painful, and nodules only appeared in the intervening skin a few days later.

At this stage the patient's general condition was still reasonably good. She had not lost weight and was eating well, despite an upper limb which was almost totally black with hundreds of melanotic nodules. Urine tests showed that large quantities of melanogen were being excreted. Three weeks later nodules appeared

in the skin between the areola and the midline, and the patient's condition began to deteriorate and she died 4 months after admission.

At the post-mortem examination it was found that the internal mammary chain of lymph nodes was infiltrated with metastatic melanoma cells, but no trace of tumour, either macroscopic or microscopic, could be found beyond this area. The sequence of events was therefore as follows:

1. Primary growth in right hand with extension to axillary glands.
2. Appearance of multiple growths in intervening areas.
3. Extension to supraclavicular glands with subsequent appearance of nodules in intervening skin.
4. Extension to areola of right breast followed by nodules in intervening skin area.
5. Extension to internal mammary glands, with subsequent appearance of nodules in intervening skin.

This type of clinical spread in the Bantu, obviously due to lymphatic embolism and retrograde permeation by tumour, suggests a revised approach to the management of the disease.

In 4 cases in which the primary malignant melanomas were situated on the foot, pre-operative radiotherapy was given. Satisfactory clinical regression was obtained in 3 cases, and local excision made possible after doses of radiation varying from 4500 r to 6000 r. Although histological examination of the excised tissue usually reveals typical melanoma cells apparently unaffected by the radiation (Fig. 7 and 8), we have not seen local recurrence in cases treated in this way. The fourth case, an elderly female, showed complete regression and macroscopic disappearance of the tumour, leaving an ulcer crater (Fig. 9 and 10). Three months later the ulcer had almost healed. When seen 5 months after discharge there was a suspicious satellite nodule, but the patient refused further treatment. In 2 of the cases the glands disappeared with radiation therapy, and in the remaining 2 were reduced sufficiently to make block dissections possible.

The clinical course therefore is less virulent than in the European and resembles to some extent the behaviour of malignant melanomas in childhood (Spitz, 1948), and it is suggested that a combined radiation-excision treatment might prove curative in early cases.

Malignant melanoma, although generally held to be radio-resistant, can occasionally be made to respond dramatically to large doses of radiation, as was illustrated in a patient with an enormous tumour in the neck.

Case 5.

This male, aged 47, was admitted with a history of a rapidly growing tumour in the neck, which had started 18 months earlier. The tumour fungated through the biopsy scar, and was of such a virulent nature that a daily increase in circumference of the neck could be noted. The patient's general condition was good, and there was no clinical evidence of secondary growths.

It was impossible to irradiate the whole mass as a single entity and a strip field technique was used. Single doses of 3000r (180 kv., 0·5 mm. Cu. + 1 mm. Al filtration) were applied to 15 × 3 cm. areas twice weekly, until the whole of the affected area had been treated. Three weeks later some regression was noted and 6 weeks after the beginning of the treatment the tumour mass was disappear-

ing rapidly. The tumour mass had been replaced by a large cavity. The sternomastoid muscle and the main blood vessels in the neck had disappeared, and the lateral wall of the larynx and the transverse processes of the vertebrae were visible. Obvious tumour tissue was present in the depths of the cavity, and this was confirmed by biopsy. The patient died nearly 6 months after admission from an aspiration pneumonia due to penetration of the larynx by the tumour, but the tumour had remained regionally localised.

CONCLUSIONS.

1. Skin cancer is relatively infrequent in the pigmented African, and its clinical behaviour differs in many respects from that generally encountered in the European.

2. In our experience malignant melanomas are as frequent in the South African Bantu as in the European and certainly more frequent than in the American Negro.

3. In the South African Bantu malignant melanomas run a less virulent course, and tend to remain localised to the regional lymphatic system.

4. The melanoma is not radiosensitive, but responds to doses of not less than 3000 r in a single application or 6000 r delivered in an overall period of 7 to 14 days. However, since the rate of dissemination is relatively slow, by making use of grids, sieves or strip fields it is possible to deliver effective doses, sufficient to eradicate the local growth in some cases.

5. A modified surgical approach for melanoma is also suggested. This consists of a wide local excision, followed after a suitable interval by a block dissection of the regional lymph glands.

6. It is suggested that in early cases of melanoma combined irradiation and surgery might prove curative, and would avoid mutilating operations, which these patients often refuse.

7. Squamous epithelioma in the Bantu almost invariably arises in sites subjected to chronic irritation or trauma, the lower extremity being involved more often than any other area.

8. The optimal treatment for these epitheliomas consists in a combined surgical procedure and specially adapted radiotherapy (grids), as the tumours are generally too large for any single conventional method.

9. Carcinomas of sebaceous gland origin have been encountered, and these were found to be radiosensitive.

10. The Bantu albino is exceedingly susceptible to cancer of all the exposed skin. The lesions appear at a relatively early age and are invariably multiple.

11. Treatment of skin cancer in the albino consists of conventional radiotherapy supplemented by podophyllin application to the pre-cancerous keratoses.

12. Since the adult albino is otherwise inevitably doomed to die of skin cancer, it is suggested that prophylaxis (sheltered indoor employment) is the only practical solution to this problem.

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THE RELATIONSHIP OF MOLLUSCUM SEBACEUM (KERATO-ACANTHOMA) TO SPONTANEOUSLY HEALING EPITHELIOMA OF THE SKIN.

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THERE is an astonishing neglect in the literature of the benign cutaneous condition described by MacCormac and Scarff in 1936, and named by them molluscum sebaceum. An alternative name, kerato-acanthoma, has since been applied, and this has the merit of preventing confusion with molluscum contagiosum, an unrelated condition. It has been the subject of only 4 papers, all by British authors, MacCormac and Scarff (1936), Musso and Gordon (1950), Rook and Whimster (1950) and Beare (1953), and does not yet appear to have reached text-books of dermatology or pathology. That this in no way reflects the incidence of molluscum sebaceum is certain. Indeed, it is not an uncommon condition, and is still very frequently misdiagnosed as squamous-cell carcinoma by both dermatologist and pathologist.

Smith, in 1934, described the occurrence in a young man of multiple primary squamous-cell carcinomas of the skin which healed spontaneously and he was unable to find any comparable case in the literature. Since that time there have been eight papers on the subject (Table II) with descriptions of similar lesions in 11 patients. Comparison of the clinical and pathological descriptions of these cases with the natural history and the structure of molluscum sebaceum at once suggests the probability that all the recorded cases of spontaneously healing epithelioma of the skin are in fact instances of molluscum sebaceum.

The following account is based on a series of 7 cases of molluscum sebaceum which after biopsy diagnosis were untreated and allowed to run their natural course to spontaneous regression. Numerous other cases have been studied, but are not included here because they were treated by radiotherapy or surgery.

Molluscum sebaceum.

Clinical features.—Some of the clinical features of the lesions in our 7 cases are summarized in Table I.

Histology.—The histological structure of molluscum sebaceum was admirably described and illustrated by Dunn and Smith (1934), though, unfortunately, under the diagnosis of " Self-healing primary squamous carcinoma of the skin." That they were not fully convinced of the correctness of the diagnosis is suggested by their statement that " It may be we are dealing with something which is not quite cancer in the ordinary accepted sense, but which cannot in its early stages be

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TABLE I.—*Cases of Molluscum Sebaceum Untreated after Biopsy Diagnosis.*

Case.	Age.	Sex.	Occupation.	Site of lesion.	Period of active growth prior to first attendance (weeks).	Total duration of lesion (weeks).	Final result.
I	50	M.	Mill foreman	Neck	3	18	Small white scar.
II	54	M.	Toolkeeper	Chest	3	15	White scar.
III	49	M.	Foreman plater	Cheek	4	16	Small pitted scar.
IV	50	M.	Labourer	Eyelid	2	7	Small scar.
V	54	M.	„	Cheek	2	8	Small depressed scar.
VI	51	M.	Foreman process worker	„	2	9	Pitted scar.
VII	69	F.	Household worker.	Face	6	14	No scar.

differentiated from cancer, either histologically or clinically." With the present knowledge of molluscum sebaceum, experience and adequate biopsy material it is possible now in a large percentage of cases to separate molluscum sebaceum from squamous-cell carcinoma.

As Rook and Whimster (1950) have described the histological changes during growth and regression of molluscum sebaceum, only the fully developed lesion will be described here. Of this stage, the structure illustrated (Fig. 2) is fully typical. In a central vertical section it is seen as an invagination or crypt containing keratin and lined by hyperplastic epidermis, which internally forms papillary projections and externally penetrates the dermis as cords and cell nests. Through a central opening, of variable size, the keratin projects on the skin surface. The surrounding epidermis, as it extends over the dome-shaped lesion, becomes attenuated and loses its rete processes. At the margin of the central pore the epithelium is acutely reflected and, inverted, runs parallel to the skin surface for a variable distance before curving to form the lateral walls and then the floor of the central crypt. Keratinization within the hyperplastic stratified squamous epithelium forming the walls of the central cavity leads to the formation of secondary, keratin-filled crypts which discharge into the main cavity (Fig. 3). Their walls form irregular, papilliferous projections into the main crypt. From the deep aspect of the hyperplastic epithelium, irregular, keratinizing cords and cells nest (Fig. 3) of progressively and rapidly diminishing size invade the surrounding dermis, destroying and engulfiing connective-tissue fibres (Fig. 4) and arrector pili muscles. At the advancing margin (Fig. 5), variation in nuclear size and intensity of staining may be pronounced, and mitoses are fairly numerous while the lesion is enlarging. In contrast to this appearance of active invasive growth are the rapidity and completeness of differentiation in the hyperplastic epithelium, which may in places even develop a stratum granulosum.

In most cases, the deep margin remains well within the dermis but occasionally, with secondary infection, penetration may be deeper. Hair follicles and sweat ducts may be engulfed in the hyperplastic epidermis and the ducts frequently show squamous metaplasia of their lining epithelium.

About the lesion there is infiltration of the dermis by leucocytes—lymphocytes, plasma cells and polymorphonuclears—amongst which eosinophilic polymorphs may be prominent. Foreign-body giant cells, grouped about keratin, may be present at this stage, but are more commonly found during the stage of regression. No inclusion bodies are present.

That the lesion of molluscum sebaceum closely simulates highly differentiated squamous carcinoma will be apparent from the histological description and illustrations. There is no single feature which will allow differentiation. With a biopsy specimen adequate in size and depth, however, a histologist familiar with the picture of molluscum sebaceum will, in most cases, be able to make a correct diagnosis. It has about it an appearance of benignity which is difficult to define but which is due probably to its regularity of structure, relatively superficial position in relation to its size and the completeness of differentiation of the hyperplastic epithelium. The clinical appearance (Fig. 1(*a*)) and a history of rapid growth over a period of 4 to 8 weeks are essential aids in diagnosis.

Spontaneously Healing Epithelioma.

Clinical features.—Table II summarizes some of the clinical features of all cases described as spontaneously healing epithelioma.

Histology.—The histological picture in all recorded cases of spontaneously healing epithelioma is identical with that of molluscum sebaceum.

DISCUSSION.

The recorded cases of multiple spontaneously healing epithelioma possess characteristics closely similar to those of molluscum sebaceum, whilst differing markedly from ordinary squamous carcinoma, single or multiple. These features will be described from the following points of view: rate and duration of growth, local and lymphatic spread, multiplicity and seat of lesions.

Rate and duration of growth.

The lesions of both molluscum sebaceum and spontaneously healing epithelioma have a short period of very rapid growth, lasting 4 to 8 weeks, followed by a stationary period and then regression to complete resolution. In untreated cases the whole process is complete in 4 to 6 months. Variations in size, duration and extent of scarring occur with secondary infection. Not only is their rate of growth and course the same, but their clinical appearance during these stages is identical. Each begins as a minute papule, which rapidly enlarges to become, at the end of the period of growth, a circular or oval umbilicated nodule of 1 to 2 cm. in diameter, which is raised $\frac{1}{2}$ to 1 cm. above the skin surface and has a keratotic centre (Fig. 1 (*a*)). About this central plug of keratin is a broad, dull red, convex and smooth-surfaced epidermal rim. With regression there is gradual flattening of the lesion, and finally the keratin plug falls off to leave a clean, slightly depressed scar.

Ordinary squamous carcinoma usually has a much slower rate of growth with progressive increase in size for several months. Although rapid growth may occur, it is usually accompanied by early involvement of the regional lymph glands. That rapidity of growth has not been accompanied by metastasis to the local lymph nodes in the spontaneously regressing cases has indeed been stressed by some of the authors. Dunn and Smith (1934) state of the lesion in their case, "It had already in the short time of 3 to 5 weeks reached a size which squamous epithelioma usually takes as many months to attain. Rapidity of growth is usually associated with early involvement of the regional glands, but this was not the case here."

TABLE II.—*Recorded Cases of Spontaneously Healing Epithelioma.*

Case.	Author.	Age.	Sex.	Age at onset.	Occupation.	Family history	Site of lesions.	Approximate duration of individual lesions (months).
1	Smith (1934)	23	M.	16	Miner	—	Face, ears, arms, thighs, legs	6
2	Dunn and Smith (1934)	42	M.	42	Sheet-iron worker	—	Thigh, leg.	—
3	Smith (1948)	58	M.	29	Coal miner	? Father	Face, ears, nose, neck, arms, legs	3
4	Ayres (1948)	63	M.	40	—	—	Face, ears, hand	—
5	Grzybowski (1950)	57	M.	49	Motor mechanic	—	Scalp, face, ears, neck, upper thorax	4 to 8
6	Sommerville and Milne (1950)	34	F.	18	Member of Armed Forces	Mother, brother, maternal aunt	Scalp, face, ears, nose, fore-arms, legs	4
7	Charteris (1951)	69	M.	23	—	? Father	Face, forearms, perineum	4 to 8
8	" "	28	M.	Before 21	Apprentice printer	Son of Case 7	Face, ears, thigh, leg	—
9	" "	43	M.	38	Ditto	" "	Face, ears, wrist, leg	—
10	" "	29	M.	13	Apprentice engineer	" "	Face, ears	—
11	Witten and Zak (1952)	49	M.	48	Leather inspector	—	Whole body—face, neck, trunk, limbs	—

Local and lymphatic spread.

Were the alleged cases of spontaneously healing epithelioma true instances of squamous carcinoma there should be good evidence of local destructive invasion and lymphatic extension, but this is lacking. In over half the recorded cases the regional glands are not mentioned and, in view of the long survival period in these cases, it may reasonably be presumed that they were not involved. For example in Smith's (1948) Case 1 there is a history of lesions for 21 years and in his Case 2 for 29 years. The patient of Sommerville and Milne (1950) had multiple lesions over a period of 22 years. Lack of lymphatic involvement was mentioned by Dunn and Smith (1934), and Grzybowski (1950) comments on "the lack of involvement of lymph nodes in spite of the 9 years' duration of the disease." In only one case, Charteris' second (Charteris, 1951), is enlargement of lymphatic glands mentioned, and these were found, histologically, to be tumour-free.

Local invasion, too, appears to have been very limited in the spontaneously healing cases, being restricted to local penetration into the dermis. Thus, Dunn and Smith (1934), "the invasive process at its height never appears to transgress the limits of the true skin." This feature applies equally well to molluscum sebaceum. Description by two authors of local tissue destruction in spontaneously healing epithelioma requires comment. Smith (1948), when recording the subsequent history of his Case 1, states that he was an irregular attender and "permitted much further destruction of the soft tissues of his face," and that he now wore a prosthesis to cover extensive destruction of his nose. This on first reading seems proof of an infiltrating and destructive malignant growth, but it must be pointed out that in the previous paragraph we are told that following treatment of a lesion of the right leg by radium, the same patient had to undergo amputation of his leg because of necrosis of the tibia. Lesions of his face had been treated by contact X-ray therapy. If this extensive destruction of the nose, requiring a prosthesis, was indeed due to squamous carcinoma, then the lack of lymphatic extension and the ultimate spontaneous healing (*sic*) make this case even more remarkable. The second author, Charteris (1951), gives the final reference to his first patient as "eventually we found that he died in November, 1945, from rapid and extensive recurrence of the anal lesion." But the last contact with the patient had been in April, 1945, when he attended hospital presumably with no recurrence of his anal lesion, and of the terminal condition there is neither biopsy nor necropsy confirmation.

Multiplicity and seat of lesions.

In all 11 recorded cases of spontaneously healing epithelioma the lesions were multiple. There was either progressive generalised extension from an initial lesion or sporadic eruption of a few lesions over many years. This feature of multiplicity seems contradictory to spontaneous healing of a malignant growth, for it is reasonable to expect that a person with such a remarkable power of control over a malignant process should have some resistance to the formation of similar malignant tumours. It is of interest that the converse holds, namely that there is no report of spontaneous healing of a solitary squamous carcinoma. That spontaneous healing should be confined to multiple primary squamous carcinoma seems contrary to the laws of probability.

It is well known that primary squamous carcinoma may be multiple, but these develop in skin which has previously undergone some pathological, "precancerous" change such as senile keratosis or dermatitis due to X-rays or arsenic. The spontaneously healing epitheliomas, on the other hand, have all developed in healthy skin. To quote Smith (1934), "photographs show how normal and 'young' the skin is, apart from the actual lesions and scars." They have displayed, therefore, not only the rarity of multiplicity itself but of multiplicity without "precancerous" lesions, and also despite the ability of the host to produce spontaneous healing.

If, as appears possible, an infective agent is the cause of molluscum sebaceum, the bodily distribution and the familial incidence in cases of spontaneously healing epithelioma might readily be explained. In both Grzybowski's (1950) and Witten's and Zak's (1952) patients the progressive spread from a lesion on the scalp to the face, ears, neck, chest and trunk is very suggestive of auto-infection. Another interesting feature in this connection is the rapid spread of the lesions under bad hygienic conditions. While 3 of these patients were prisoners of war there was an enormous increase in number of their lesions. With regard to the familial incidence noticed in 3 of the 11 patients, the following members of each family had similar lesions:

1. Somerville and Milne (1950): the patient's mother, brother and maternal aunt.
2. Charteris (1951): the patient's father and three sons.
3. Smith (1948): Case 2: the patient's father.

This has been stated to be due to a familial inherited tendency towards squamous carcinoma of the skin. Yet what would appear to be a directly opposed factor—ability of the same individual to produce spontaneous healing—is also ascribed to an inherited factor. However, if all the cases described as spontaneously healing epithelioma were in fact molluscum sebaceum the explanation is much simpler, and there is no need to search for an inherited diathesis. Infectivity would account for spread within a family and spontaneous healing would be the natural termination.

SUMMARY.

Attention is drawn to the close similarity, in structure and natural history, between molluscum sebaceum and the cutaneous lesions described in the literature as multiple spontaneously healing epithelioma. It appears probable that the two conditions are identical, and that there is no acceptable record of spontaneous regression of a squamous carcinoma of the skin.

Dr. Hugh Gordon has very kindly allowed us to make use of the clinical records of the patients with molluscum sebaceum. The photomicrographs are by Mr. L. A. Cowles from histological sections by Mr. C. G. Chadwin.

Since completion of this paper two other relevant communications have appeared. Currie and Smith (1952), under the title "Multiple primary spontaneous-healing squamous-cell carcinomata of the skin", describe 2 new cases. They maintain that the lesions they describe are unrelated to molluscum sebaceum. Whittle and Lyell (1952), in commenting on a case of molluscum sebaceum which

they demonstrated at the Royal Society of Medicine, " wonder if Dr. Ferguson Smith's cases of multiple self-healing epithelioma, in spite of their familial tendency, are really very different from these kerato-acanthomata ".

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EXPLANATION OF PLATES.

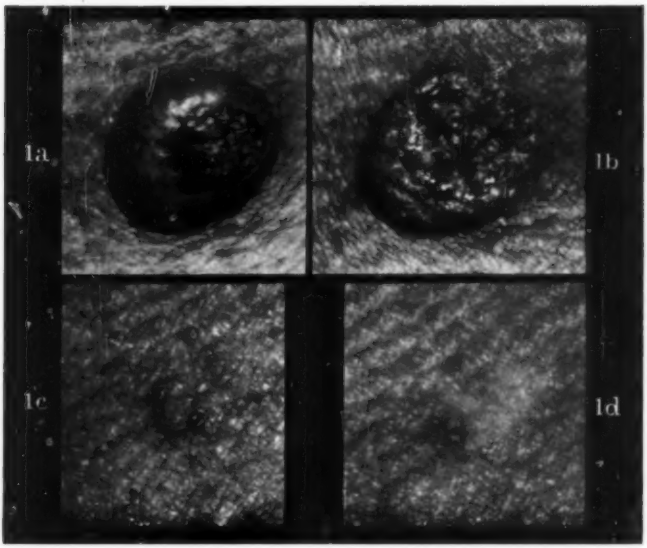
FIG. 1.—Molluscum sebaceum (Case 1) undergoing spontaneous regression following biopsy. × 2. (*a*) Typical lesion before biopsy. (*b*) 3 weeks after biopsy. (*c*) 11 weeks after biopsy. (*d*) Residual scar 23 weeks after biopsy.

FIG. 2.—Molluscum sebaceum (Case 2). Central vertical section showing the central keratin-filled crypt bounded by hyperplastic epithelium. H. & E. × 7.

FIG. 3.—Molluscum sebaceum (Case 2). Vertical section near periphery of same lesion as Fig. 2 showing secondary crypts and the papillary projections forming their walls. Van Gieson. × 10.

FIG. 4.—Molluscum sebaceum. Elastic fibres engulfed by the advancing margin of hyperplastic epithelium. Verhoeff's elastic. × 90.

FIG. 5.—Molluscum sebaceum (Case 1). Epithelial cell nests penetrating into the dermis, which is infiltrated by chronic inflammatory cells. H. & E. × 55.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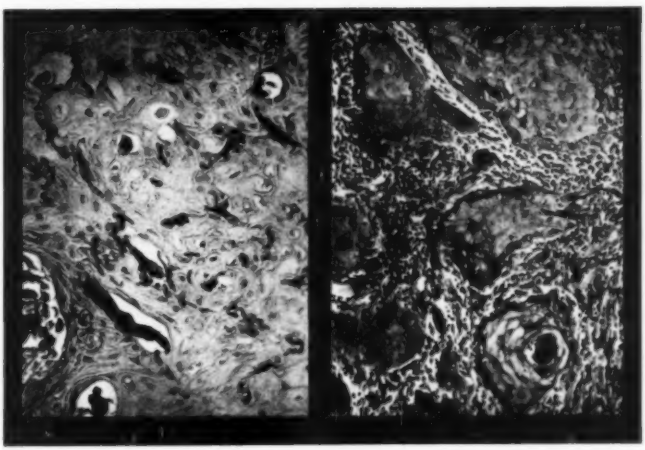

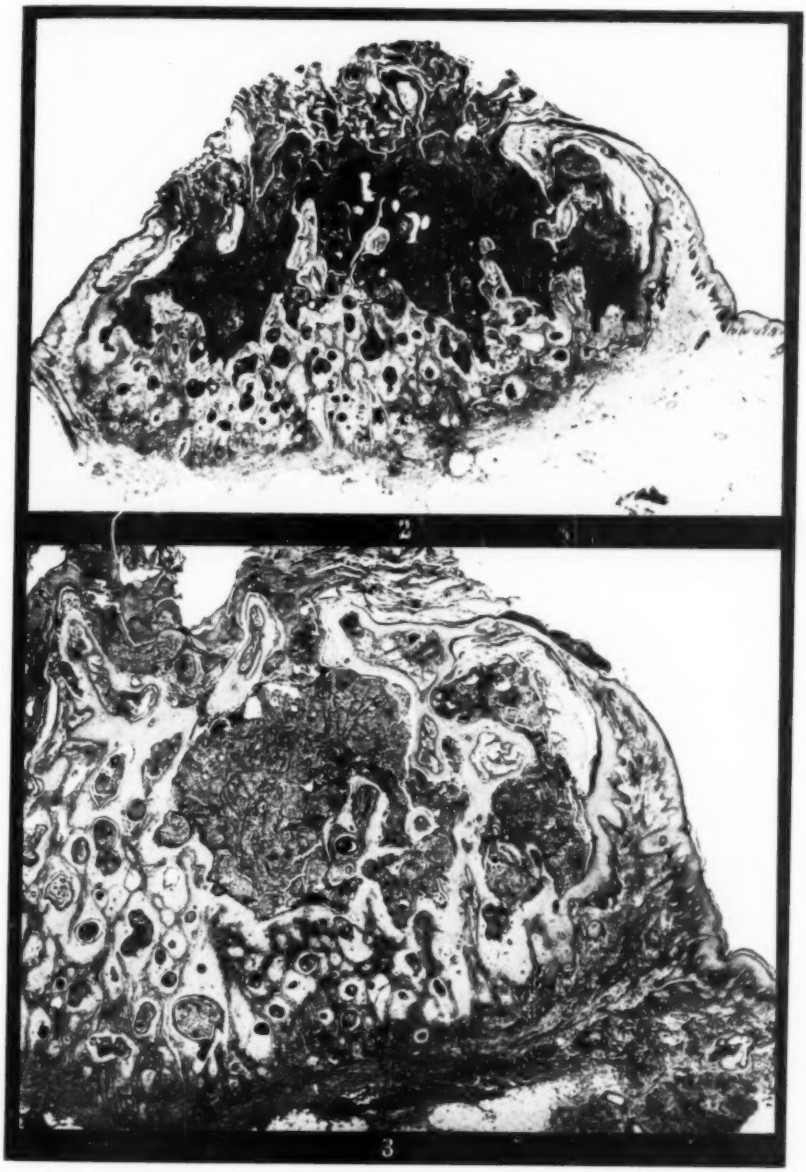

Fouracres and Whittick.

DUODENAL CARCINOMA WITH NEOPLASTIC TRANSFORMATION OF THE UNDERLYING BRUNNER'S GLANDS.

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CARCINOMA of the duodenum is undoubtedly very rare: Eger's (1933) analysis of the literature showed that, of 350,286 autopsies, it was recorded in only 126 (0·033 per cent).

The following case is considered worthy of recording, firstly because it shows conclusive evidence of a field of origin involving both mucosal epithelium and Brunner's glands, and secondly, because of its occurrence in conjunction with another primary tumour of the alimentary tract.

Case Report.

R. McB—, a male, aged 82 years, first visited the Royal Cancer Hospital in October, 1949, because of a painless "roughening of the gum" on the right side of the mouth which had been present for about one year and, for the last 3 months, had caused difficulty in eating and swallowing. Further questioning elicited no other symptoms referable to the gastro-intestinal tract.

On examination he was found to have a deeply ulcerated and extensive tumour involving the posterior margin of the right buccal mucosa, both upper and lower alveolae posteriorly and the right side of the soft palate supero-medially. Biopsy examination showed this to be a partially keratinizing squamous-cell carcinoma. He died a month later.

Necropsy.

There was confirmation of the buccal tumour. Routine examination of the duodenum disclosed a centrally ulcerated, circular, indurated lesion (1½ cm. in diameter) in its second part, 1½ cm. proximal to the papilla of Vater. A fine probe was passed easily into the ampulla and thence on into the common bile duct which, although close to the floor of the ulcer, was not involved (Fig. 1). Macroscopically the lesion closely resembled a typical benign, chronic duodenal ulcer with a thickened, oedematous edge and a dense sclerotic base (Fig. 2). An accessory pancreatic duct opening into the duodenum was searched for without success. The remainder of the intestine, both small and large, showed no abnormality. No metastases were present in the liver, lungs, kidneys, adrenals, testicles, brain, skin or bones (only the vertebral bodies, ribs, iliac crests and sternum were examined).

Histology.

Numerous cross-sections of the duodenal lesion all showed a well-differentiated columnar-cell adenocarcinoma, which extended laterally in all directions and deeply to involve the peri-duodenal areolar tissue for a maximum distance of 7 mm. beyond the muscle coat which, in the floor of the ulcer, had only been partly replaced by tumour and fibrous tissue (Fig. 2).

Owing to the time elapsing between death and necropsy (13 hours) most of the superficial epithelium of the duodenal mucosa had been destroyed, but that present in the crypts on both sides of the ulcer showed evidence of actual neoplastic transformation, with histological changes varying from nuclear pleomorphism and hyperchromatism of the cells *in situ* to actual invasion of the underlying submucosa and muscularis by well differentiated adenocarcinoma (Fig. 3).

As the lesion was approached from all sides the Brunner's glands showed ever-increasing hyperplasia until, close by the edge of the ulcer, there was undoubted evidence that they, together with the epithelium in the adjacent crypts of Lieberkuhn, had undergone malignant change, as shown both by cytological alterations and by actual invasion of the surrounding tissues (Fig. 5 to 8 inclusive). The typical foamy, ballooned, mucus-secreting acinus cell of the nearby glands (Fig. 4), unstained by eosin but giving a deep magenta colour with Schiff's periodic acid-fuschin stain, showed a gradual change to a well-defined, high columnar cell now staining brightly with eosin, but either completely devoid of Schiff positive material or with it present only in small amounts aggregated near the free, inner edge of the cell.

There was no convergence of the muscularis mucosae and muscularis at the ulcer edges; in fact they actually diverged, being forced apart by masses of tumour and fibrous tissue. A few small arterioles present in the floor and edges of the ulcer showed no evidence of endarteritis.

At one point the tumour had penetrated the periphery of the pancreas for a distance of approximately 2 mm. only. There was no involvement of the common

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EXPLANATION OF PLATES.

FIG. 1.—Showing relation of tumour to ampulla of Vater through which a probe has been passed into the common bile duct. Natural size.

FIG. 2.—Cross-section of tumour showing, *inter alia*, retention of muscularis in floor of ulcer and absence of its union with the muscularis mucosae. H. & E. × 4.

FIG. 3.—Showing muscularis invaded by the tumour. H. & E. × 135.

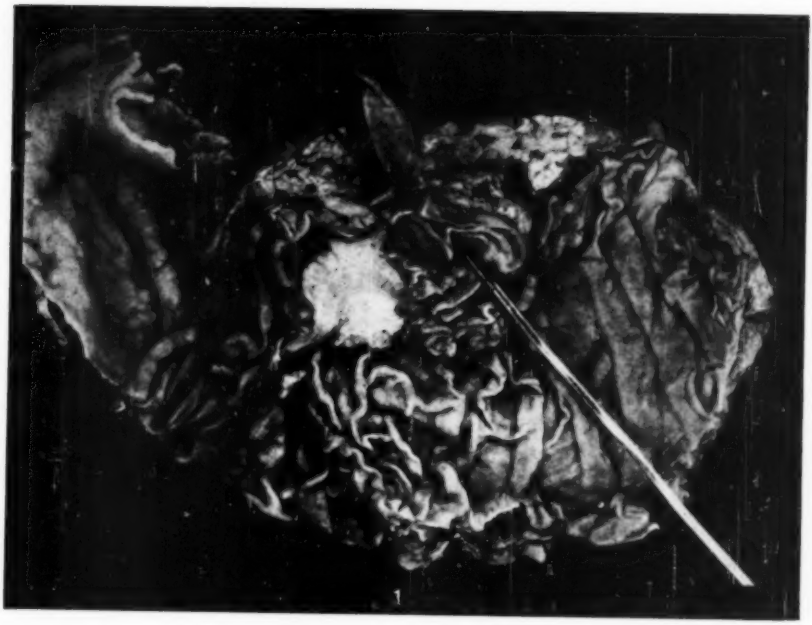
FIG. 4.—Showing, for purpose of comparison, a normal Brunner's gland in nearby mucosa. (Post-mortem change has not appreciably altered its characteristic appearance.) H. & E. × 145.

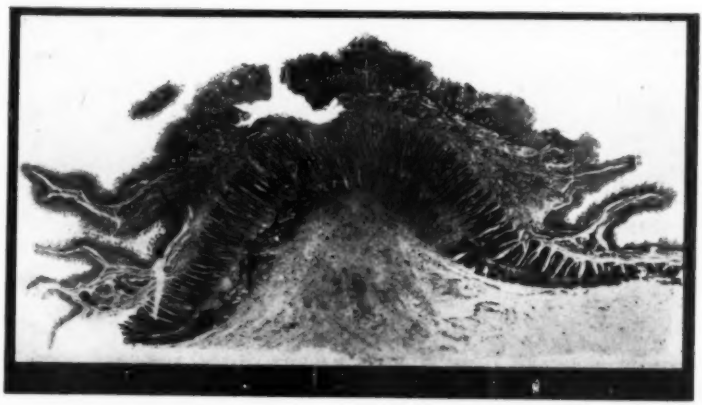
FIG. 5.—Higher magnification of area marked in Fig. 2, showing transformation of hyperplastic Brunner's glands (on right side) into an invasive adenocarcinoma (left side). H. & E. × 30.

FIG. 6.—Higher magnification of area within right-hand square in Fig. 5, showing both hyperplastic Brunner's glands (on right side) and atypical glandular acini (on left side). H. & E. × 120.

FIG. 7.—Higher magnification of central square in Fig. 5, showing a further stage in the neoplastic transformation. H. & E. × 120.

FIG. 8.—Higher magnification of left-hand square in Fig. 5, showing invasion of the submucosal connective-tissue strata by adenocarcinoma which has, on left-hand side, lost all resemblance to normal Brunner's gland acini. H. & E. × 120.





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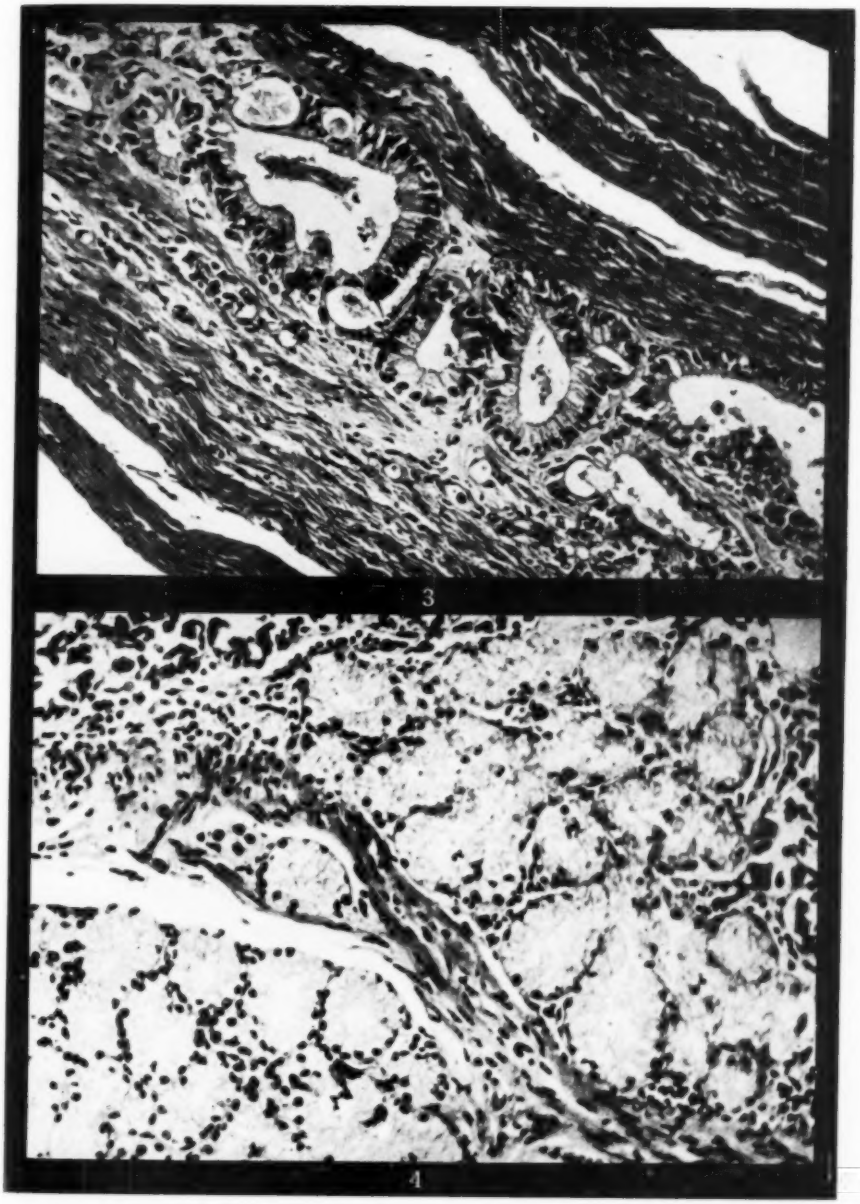

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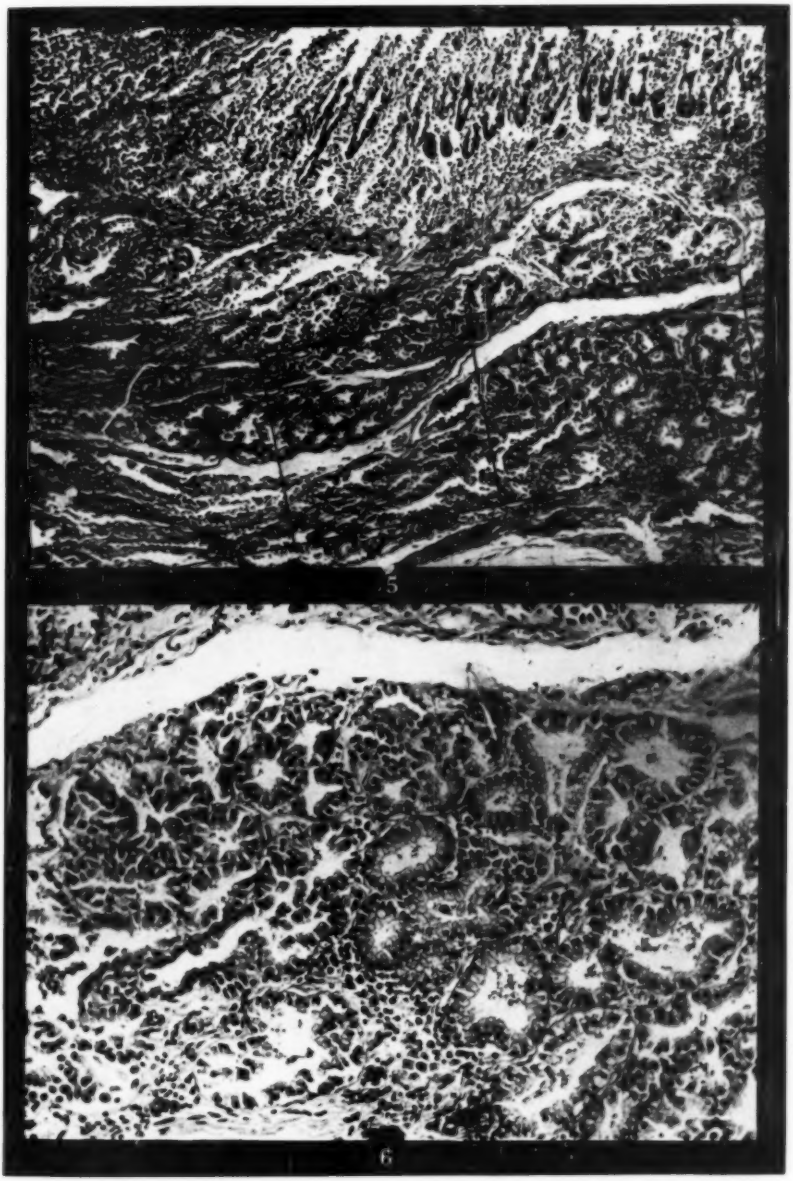

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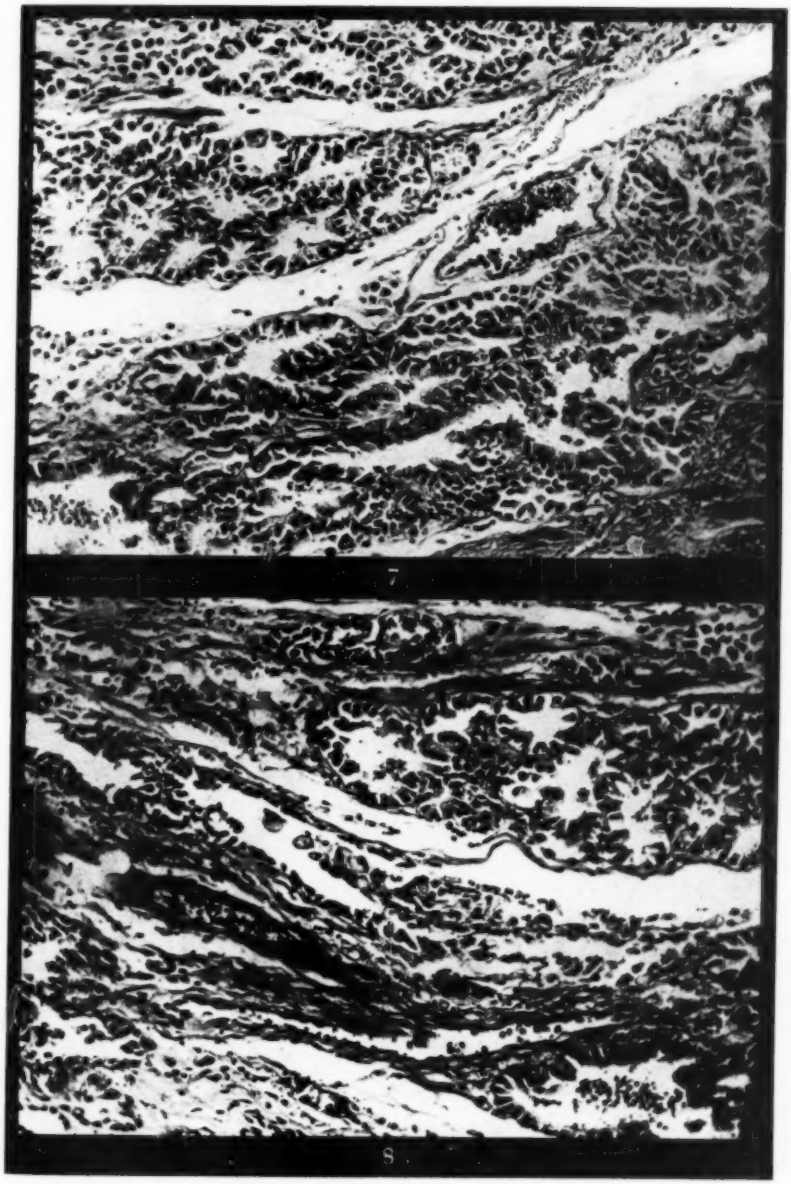

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bile duct, ampulla (or papilla) of Vater or three small, chronically-inflamed lymph nodes in the region of the tumour. Random sections of the lungs likewise showed no metastases.

DISCUSSION.

As duodenal carcinomata are reported to be most frequently situated close to the papilla of Vater (Macindoe, 1940 ; Rolleston, 1901), where both the pancreas and common bile duct lie in close proximity, it is of the utmost importance to satisfy very rigid criteria before concluding that a tumour has, in fact, arisen primarily from the duodenal mucosa.

The adenocarcinoma described above showed maximal involvement of the mucosa with spread in all directions from the central ulcer and a definite zone of transition from normal to neoplastic epithelium : that is, the field of origin was still present. Finally the necropsy findings excluded the possibility of its being a metastasis from a nearby or distant organ (the buccal tumour has a characteristic and totally different histological structure). Hence there can be no reasonable doubt that it is primarily duodenal.

Histological appearances clearly indicate that the initial neoplastic change has involved the epithelium throughout the whole thickness of the mucous membrane, including Brunner's glands. The author has been unable to find any other recorded case in which these glands were actually involved in the field of origin, although Stewart and Lieber (1937) did note in their Case 3 that those nearby were hyperplastic.

As there is no endarteritis of vessels in the floor or edge of the lesion and neither complete destruction of the underlying muscularis nor fusion of it with the muscularis mucosae (Fig. 2), the possibility of neoplastic change in a pre-existing benign, chronic ulcer is very remote. Furthermore, such innocent lesions are far more common in, and, in fact, almost confined to, the first part of the duodenum.

SUMMARY.

A case of multiple primary cancer in an elderly male is described, the lesions being a keratinizing squamous-cell carcinoma in the floor of the mouth and an early symptomless adenocarcinoma of the second part of the duodenum.

The duodenal tumour showed a field of origin involving both mucosal epithelium and Brunner's glands.

Evidence is presented to show that the lesion is unlikely to have arisen in a benign, chronic duodenal ulcer.

An extensive search of the literature has failed to reveal a similar case with involvement of the Brunner's glands in its field of origin.

I am indebted to Mr. M. H. Harmer for permission to report the case, to Miss J. Hunt for the photograph of the specimen, to Mr. F. E. Speed for the microphotographs, and to Mr. C. G. Chadwin for the histological work.

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A NEW THEORY ON THE CANCER-INDUCING MECHANISM.

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RECENT research in genetics and pathology has shown an amazing consistency between the agents causing mutations and those causing—or contributing to the development of—cancer. One of the more prominent theories, which since the 1920's has been advocated by Bauer (1949), Strong (1949) and others, claims that the original cancerous cell is nothing but an ordinary cell affected by genetic mutation of some kind. One of the main objections to this theory has been that it does not explain the age variation of the cancer frequency. In reply, Bauer (1949) contends that the mutated cells apparently remain latent during a long period until the new qualities become evident and the phenomenon can be diagnosed as cancer. Bauer has found this period of latency to average 9 years for X-ray cancer, 12 years for paraffin cancer, 18 years for aniline cancer and 40 years for seaman's cancer (caused by solar radiation). The early occurrence of sarcoma and of leukaemia, however, does not conform to the idea of a latent period. Moreover, it appears somewhat unreasonable to suppose that the length of the latency period would depend upon whether the mutation is caused by sun-rays or by X-rays. With most forms of carcinoma, furthermore, the frequency at different ages is such that we are compelled to consider average latency periods of 70 years or more, in order to explain the actual age frequency curve of cancer mortality in man. These facts still make it difficult for many of the most competent authorities in the field to accept the mutation theory without reservations.

Dahlberg (1943) has advanced a completely different explanation concerning the relationship between age and cancer. He expressed the opinion that malignant tumours develop increasingly easily with rising age. This implies that, for the development of tumourous cells, it is necessary for a certain number of cellular divisions to have taken place, between each of which there has been a certain period of time. All experience concerning the cancerous influence of chronic irritations promoting cellular divisions seems strongly to support this theory. The question then arises of the nature of the process taking place in the cells during the intervals between their various divisions. According to the theory advanced in 1934 by Timoféeff-Ressofsky, Delbrück and Zimmer (1935) and discussed by Schroedinger (1944), mutations constitute such a process, which goes on incessantly in every large group of cells. Mutations are caused, according to this theory, by the normal molecular collisions in connection with the movements constituting temperature, as well as by other kinds of energy supply originating from, for example, X-rays.

If a large enough number of cells is allowed a sufficiently long period of time, gene mutations will necessarily occur in some of them. If the cells propagate—in other words, if the tissue in question grows or renews itself—it is possible that the mutated cells will multiply in great number. In this case it is probable

that some of the already mutated cells will mutate again and thus become provided with two abnormal genes. This process of mutation, propagation, mutation and so on may, of course, continue incessantly during the life of the individual. If the propagation of cells is rapid, the whole process will take place more rapidly ; and if the cells are influenced by mutation agents such as X-rays or mustard gas, there will also be an acceleration of the process as a whole.

Thus, these facts regarding the relationship between age and the incidence of cancer make necessary a modification of Bauer's (1949) fascinating mutation theory and recognition of the improbability that a single mutation causes the first cancerous cell. Evidently, as suggested independently by Muller (1951) and by Nordling (1952), several successive mutations in the same cell, probably about seven in the case of human cancer, would be necessary. The number of mutations required for experimental cancer in mice may be less, as suggested by the investigations of Iversen and Arley (1950). Obviously, we need not assume that any seven mutations will cause cancer. Only mutations which increase the ratio between cellular divisions and cellular loss in a positive direction in the environment in question may be expected to have this effect. According to Fischer (1930), the short average life-span of cancerous cells is their dominant feature, and that by which all their other qualities can be explained. Their rapid propagation, which exceeds their high death-rate, their ability to ferment sugar on a large scale and to disintegrate heterogeneous proteins, constitute normal cellular responses to a high mortality among adjacent cells. It may be added that the frequent variability in the number of chromosomes among cells from the same tumour is also a " normal " feature in the sense that the same phenomenon also occurs in certain normal tissues, as shown by Therman and Timonen (1951). Thus, some or most of the mutations required to start an incessant self-stimulating propagation among a group of cells might consist of any of the forms of mutation that weaken the cell, and make it more liable to die from even minor disturbances.

If cancer were caused by one mutation only, it might be expected to be equally common among persons of different ages after an age corresponding to the length of the latency period, if this period were interpreted as the time required by the first cancerous cell to multiply in such degree as to be diagnosed as cancer. The latent period of X-ray cancer (9 years according to Bauer (1949)) and the incidence of sarcoma in early childhood indicate that the latent period for cancer in general is, in reality, to be counted in years or months rather than in decades. If two mutations were required, the frequency of cancer should increase in direct proportion to age, because cells once mutated (which are able to mutate a " second " time) evidently increase in number in direct proportion to age. If three mutations were required, a cancer frequency proportional to the second power of age might be expected, with four mutations to the third power of age, and so on. This implies that the hypothesis of successive mutations as the cause of cancer can be substantiated only if there is a coincidence between the frequency of cancer and a certain power of age. If, on the other hand, the frequency of cancer actually ceases to increase after a certain age, the hypothesis must be rejected.

Actually, the cancer statistics from several countries indicate a falling cancer death-rate from about 70 years of age and upward. In other countries, however, there is a continuous increase in the cancer death-rate from lower to higher ages, as far up in age as the statistics go. It now appears that the former situation

prevails in countries with a high percentage of deaths due to "senility", "other and unknown causes" and the like, whereas the latter is the case in countries with detailed and reliable statistics, e.g., the United States, New Zealand, Australia, Great Britain and other western European countries. It can be shown, as by Nordling (1952), that the cancer frequency curve of a population with undependable vital statistics will be restored to the same shape as that of the populations with accurate statistics if, as a rule, about one-fourth of the deaths due to such causes as "senility" are added to the registered cancer deaths.

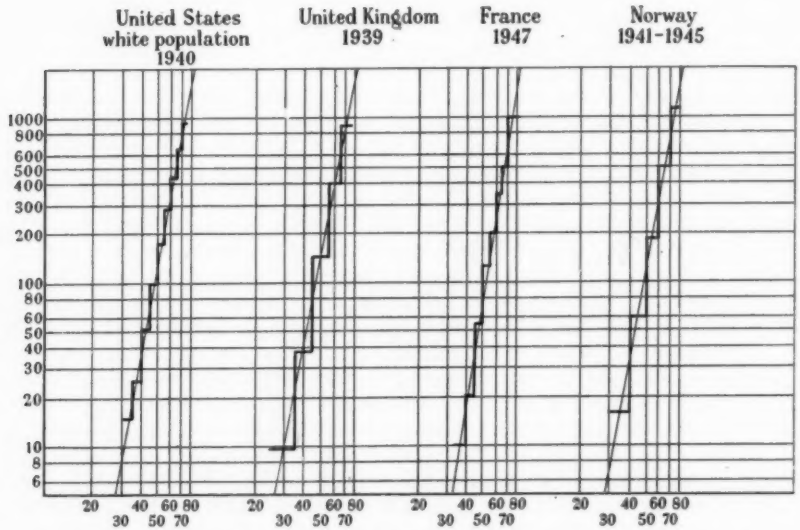

FIG. 1.—Diagram drawn to double logarithmic (log/log) scale showing the cancer death-rate (in the case of the United Kingdom, the carcinoma death-rate) in males at different ages. Deaths per 100,000 males are shown on the vertical scale, age figures on the horizontal scale.

However, the very highest age-groups, which are always numerically small and certainly highly selected, can hardly be taken into consideration. This is because these individuals are usually already very frail and likely to die from any trivial cause, irrespective of whether or not they have cancer in a more or less developed stage.

Thus, it seems probable that, in reality, the age-frequency relationship for cancer follows the same general rule everywhere in the civilized world, although reliable examples can be given in only a limited number of cases. In these cases it is obvious, as is shown in Fig. 1, that the cancer mortality in males increases according to a certain power (the sixth) of age. The scales of the diagram are logarithmic, and consequently every curve of the type $y = x^a$ becomes a straight line. It is worth noting that deaths from other causes follow different age curves, and that the sixth power curve is characteristic particularly for

cancer. With regard to cancer among females, it is necessary to distinguish between cancer in the specificially female organs, of which the increase is fairly small above the age of 45, and cancer at other sites, of which the frequency seems to increase according to the sixth power of age both before and after the forties, but not during the decade of the menopause, when the increase is smaller.

It seems possible that altered hormonal conditions in connection with the menopause might play a part in determining the actual cancer frequency curve among women. Obviously, more slowly operating hormonal variations might be present in both men and women. It is therefore by no means certain that the entire increase in the incidence of cancer with age is to be explained by means of the multiple mutation mechanism. It is possible that a small proportion of the increase is due to a lesser degree of hormonal inhibition of growth of potentially cancerous cells in an older organism than in a younger one. The writer is not, however, aware of any evidence definitely substantiating such a variability of natural cancer inhibition with age.

During childhood and adolescence cancer is rare, but it occurs considerably more often than the sixth-power-of-age rule would allow. In these cases it is mainly a question of sarcoma, leukaemia and other non-epithelial forms of cancer. Such a condition does not, however, imply any contradiction of the hypothesis of successive mutations as the cause of cancer. Since it is unlikely that mutagenic noxae reach the bones and other internal tissues, it therefore seems more reasonable in the case of sarcoma to presume spontaneous mutations (i.e., mutations due to normal molecular collisions) and radiation mutations as the causative factor. Such mutations may, of course, occur already in the foetal period. In view of the extremely rapid growth during this period, it is not surprising that multiple mutations occasionally have time to occur, giving rise to infantile or even foetal sarcoma. The infrequency of malignant tumours in the striated muscles, despite their large volume, may have some connection with the fact that this kind of tissue is composed of multinuclear cells.

According to the theory put forward in the present paper, cancer is caused by mutations multiplied and accumulated usually through large-scale proliferation of cells. It is easy to explain on this basis such facts as the (sometimes inherited) high susceptibility to cancer of certain organs, especially under experimental conditions. This is because every stimulant causing—or congenital capacity characterized by—a high proliferation rate also increases the number of mutated cells and thus the probability of cancer. Also the high susceptibility to cancer of benign tumours is easy to understand in this way.

The facts and theories advanced here demonstrate, among other matters, the importance of having reliable vital statistics in order to elucidate casual relationships in the pathogenesis of cancer. The extent to which cancer statistics have been improved in several countries during the past decade is striking. This opens up important fields for research. Among the multitude of still unexplained statistical data concerning cancer, mention might be made of the great difference in the cancer frequency between Whites in the north-eastern United States and among those living in the southern part of the country, as well as between the Negroes in these two parts. The fact that environment rather than race appears to be responsible for these differences is encouraging, since it indicates the possibility of greatly reducing the incidence of cancer.

SUMMARY.

The theory is put forward that the cancerous cell contains not one but a number of mutated genes. The occurrence of such accumulations of mutations may be expected to increase according to a certain exponent of age, as well as according to the increase of cell proliferation. Cancer statistics also show that the frequency of carcinoma increases according to the sixth exponent of age in males.

The unexpectedly high incidence of internal neoplasms in childhood is explained by the high frequency of cell division in the foetal stage. Other high cancer incidence rates in particular organs may be explained on the basis of exposure of the tissues either to mutagens or to agents increasing cell proliferation.

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A STUDY OF THE DEVELOPMENT OF MAMMARY TUMOURS IN HYBRID MICE.

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THE test mice most commonly used for ascertaining the presence of the mammary tumour agent are susceptible hybrid female mice obtained by mating low-breast-cancer-strain females to high-breast-cancer-strain males. In these test mice a low incidence of spontaneous mammary tumours was frequently recorded (Bittner, 1939*b*; Murray and Little, 1939; Andervont, 1940; Gardner, 1941; Dmochowski, 1944*a*), and was frequently found to be higher than that of their maternal parents (Andervont, 1945*a*). Of even greater interest was the high tumour incidence reported in the progeny of susceptible low-cancer-strain females mated to high-cancer-strain males which had been subjected to forced breeding—that is, bearing a number of litters in quick succession (Andervont, 1945*b*). A similar high incidence of breast cancer was also observed in forcibly-bred hybrid progeny of low-cancer-strain females of low susceptibility to breast tumours mated to high-cancer-strain males (Bagg and Jacksen, 1937; Strong, 1943). Attempts to discover the mammary tumour agent in mammary tumours of some of these hybrids proved unsuccessful (Andervont, 1945*b*). A number of possibilities was suggested (Andervont and Dunn, 1949) to explain the appearance of breast cancer in hybrid females: presence of a weak agent or small quantities of it or even a different agent in low-cancer-strain female mice; transmission of the agent from the male parent or influence of the more susceptible genetic constitution of this parent; hormonal stimulation overcoming the absence or small quantities of the agent; contamination; even *de novo* origin of the agent.

In connection with the initially recorded observations on tumours in hybrid mice, an investigation was carried out in an attempt to obtain a high incidence of mammary tumours in hybrid mice bred in the laboratory, with a view to ascertaining the respective parts played by genetic constitution, hormonal stimulation, and the agent in the development of mammary cancer in these mice, obtained by mating C57 Black low-cancer-strain females of low susceptibility to the agent to RIII agent-harbouring high-cancer-strain males. It was also planned to study the inheritance of mammary tumours in the progeny of these hybrid females obtained by brother-to-sister matings.

Preliminary findings of this study have already been reported (Dmochowski, 1949*a*, 1949*b*, 1950*a*, 1950*b*, 1951*a*, 1951*b*). Biological tests for the presence of the agent in some of these tumours as well as a study of the microscopical appearance of breast cancer in these mice are now complete, and a full account of this investigation is now possible. During the intervening years, since the present

experiments were started, the problem of the origin of breast tumours in hybrid mice of various derivations has also engaged the attention of several other investigators (Foulds, 1947, 1949; Andervont and Dunn, 1948*a*, 1948*b*, 1949, 1950*b*; Andervont, 1950*a*; Bittner, 1952*a*; Mühlbock, 1952). Therefore, a comparison of the observations in the present study with those of other investigators will also be presented.

MATERIAL AND METHODS.

The experiments were started in October, 1947, by establishing a breeding colony of thirty C57 Black low-cancer-strain females and ten RIII high-cancer-strain males. The C57 Black strain mice originated from the Laboratories of the Imperial Cancer Research Fund and have been under the writer's care since 1946. Spontaneous mammary tumours have not been recorded in this strain since its establishment in the Imperial Cancer Research Fund Laboratories in 1941. The ancestors of the C57 Black strain females used in the experiments were all free of mammary tumours. A subline of C57 Black strain is now established in which a number of mammary tumours has been recorded by the author. This subline comes from one of the original three litters brought from the Laboratories of the Imperial Cancer Research Fund. The progeny of these litters has been kept separately, and no breast tumours have so far been recorded in the descendants of the other two litters. The description of the subline in which tumours have appeared will be the subject of a separate communication. The RIII strain mice were also obtained from the same source and maintained since 1946. The tumour incidence of 83 per cent at an average age of 8·5 months in breeding females (Dmochowski and Gye, 1943) has with small variations remained approximately the same. It was known that mice of high-cancer strains derived from the fourth or later litters develop a higher incidence of breast cancer than those from the first two litters (Bittner, 1942), and that the appearance of an active agent in mice deprived of it by foster nursing had been recorded in the progeny obtained from the third or later litters (Bittner, 1943). Hybrid progeny of the C57 Black strain females was therefore taken only from the fourth or later litters for the experiment, mice from earlier litters being used for various other purposes. The (C57 × RIII)F_1 hybrid females which were litter mates were divided into two groups. Only two hybrid females were taken from each of the C57 females mated to RIII strain males, if possible from the same or following litters. Whenever two females were not available from the same litter, the mice from later litters were included in the control group. In this way two groups, each comprising thirty (C57 × RIII)F_1 hybrid females, were obtained. Mice of the control group were allowed to breed in a normal way and mice of the experimental group were forcibly bred by removing their litters as soon as they were born. Mice of one of the later litters from fifteen hybrid females in this group and of one litter of a female from the control group were saved for brother to sister matings. These mice in turn, after saving one litter, were forcibly bred and this procedure was followed for several generations. The progeny of six (C57 × RIII)F_1 hybrid females, which had been subjected to forced breeding, was maintained for six generations and the progeny of nine additional (C57 × RIII)F_1 hybrids, treated in a similar manner, for only three generations. The descendants of a (C57 × RIII)F_1 hybrid female, which had been normally bred, were maintained by normal breeding for five generations.

In addition a number of litters from several C57 × RIII hybrid females was secured after the development of a mammary tumour, in an attempt to ascertain any difference in the tumour incidence and the presence of the agent in mice born before and after their mothers developed a tumour. The progeny of mice born before and after the development of breast cancer was also kept for observation of any difference in the incidence of mammary tumours.

Biological tests of twenty-three breast tumours which developed in (C57 × RIII)F_1 hybrid females and in their progeny were carried out for the presence of the mammary tumour agent. The tumours, after securing a part for histological examination, were frozen and desiccated as previously described (Dmochowski, 1946). After storage in the ice-chest for varying periods of time according to the availability of test mice, the desiccated tumour tissue was ground in a mortar and resuspended in distilled water in a proportion of 1 : 10, and then usually further diluted to a volume according to the number of test mice available, so as to give daily each animal 1 ml. of extract intraperitoneally. The number of injections and the amount of material given at each injection varied according to the total amount of tissue available. The intention always was to give as much material as was available. (C57 × A)F_1 hybrid females, 4 to 5 weeks old, were used as test mice. All test mice were forcibly bred by removing the first three litters within 24 hours of birth, and then allowed to breed in a normal way. Spontaneous mammary tumours have not been observed in (C57 × A)F_1 hybrid females after similar treatment (Dmochowski, 1944*b*, 1945*a*), and the total tumour incidence in all these mice, so far used, is below 3 per cent. The test mice as well as all other mice were maintained under similar conditions on a diet of " rat-cake " cubes, biscuits, and oats, and given an unlimited supply of tap-water. Intermittent outbreaks of enteritis during the later part of the experiment were successfully controlled by streptomycin supplied in a final concentration of 0·025 per cent in drinking water. The number of animals kept in one cage was six in the case of the test mice, and from two to four in the case of all other mice.

Histological examination of all tumours, whenever possible, was carried out. Tumours which were not examined microscopically were excluded from the present study. The tissues were fixed in Bouin's fluid and stained with haematoxylin and eosin.

RESULTS.

No mammary tumours were observed in any of the (C57 × RIII)F_1 hybrid females which had been bred in a normal way, although they lived to an average age of approximately 19 months (Table I). One of the females developed a

TABLE I.—*Tumour Incidence in Normally-Bred (C57 × RIII)F_1 Hybrid Females.*

Number of mice alive at earliest tumour appearance.	Earliest tumour appearance in forcibly bred hybrids (days).	Mice developing tumours. / Mice dying without tumours. Age in months.										Number of tumours.	Number of mice dying without tumours.	Average age of mice dying without tumours (days).
		12.	14.	16.	17.	18.	19.	20.	22.	23.	26.			
		0	0	0	0	0	0	0	0	0	0			
29	290	–	–	–	–	–	–	–	–	–	–	0	29	559
		1	1	8	1	5	3	2	4	2	2			

(372–794)

tumour which on histological examination was found to be a lymphosarcoma. The average number of litters born to these hybrid females was three, and varied from two to seven. The number of breast tumours which developed in their litter mate (C57 × RIII)F_1 hybrid females, which had been forcibly bred, is shown in Table II. The number of litters born to these hybrids varied from four to ten and the average number was 6·4. Among these hybrids, the tumorous females had an average of 6·5 litters and the tumour-free females 6·4 litters. There was no difference therefore in the number of litters born to these two types of females which had been forcibly bred.

None of the C57 Black strain mothers, mated to RIII strain males and bred in a normal way, developed mammary cancer, although they lived to an average age of 19 mouths. Twelve C57 Black strain females, which were litter-mates of some of the C57 females mated to RIII strain males, were forcibly bred with their own litter-mate males, and none developed breast cancer, although they had an average of 5·2 litters and lived to an average age of 18 months. At the same time a number of other low-cancer-strain female mice, ten C strain, supplied by Dr. H. B. Andervont, ten JK strain, supplied by Dr. L. C. Strong, eleven P strain and five Y strain, supplied by Dr. C. C. Little, were also forcibly bred with their own strain males. In none of these females was mammary cancer observed. Their average life-span with the average number of litters born shown in parentheses was: 19 months (7·4), 20 months (8·2), 18 months (5·4), and 17 months (5·6) respectively.

The appearance of mammary tumours in some of the (C57 × RIII)F_1 hybrid females following forced breeding could be explained by the transmission of small amounts of the agent by their C57 Black strain mothers after mating to RIII strain males, as originally suggested by Foulds (1949), although none of the C57 females developed breast cancer, and further by activation of the agent in their susceptible hybrid progeny by increased hormonal stimulation. The observation that none of the C57 strain females developed mammary tumours after mating to C57 strain males and forced breeding points against the possibility of C57 Black strain females harbouring a weak or attenuated mammary tumour agent. It is now well known that various sublines of C57 Black strain differ considerably in their susceptibility to the agent. C57 strain mice of the subline of the present experiments, when foster nursed by RIII strain females, developed an incidence of mammary cancer of only 11 per cent (Dmochowski, 1948). This low susceptibility was probably responsible for the failure to induce mammary cancer in mature C57 mice of this subline even by a combined action of large doses of material containing the agent and forced breeding (Dmochowski, 1948). Again this low susceptibility may also have been responsible for C57 mice of this subline remaining tumour-free after mating to RIII high-cancer-strain males as well as for their hybrid progeny, bred in a normal way, not developing mammary cancer. A further indication against the possibility of C57 Black mice of this subline harbouring a weak agent or in small quantities was provided by the absence of the agent in mammary tumours induced in both virgin and breeding C57 females by treatment with methylcholanthrene (Dmochowski and Orr, 1949). At least it can be stated that even treatment with methylcholanthrene combined with breeding failed to reveal either a weak agent or a strong agent on a genetic background of low susceptibility in C57 Black mice of the subline used in the present study.

TABLE II.—*Tumour Incidence in Forcibly-Bred (C57 × RIII)F_1 Hybrid Females.*

Number of mice alive at the earliest tumour appearance.	Earliest tumour appearance.	Mice developing tumours. / Mice dying without tumours. Age in months.												Number of tumours.	Tumour incidence (%).	Average tumour age (days).	Number of mice died without tumours.	Average age of mice dying without tumours (days).
		10.	11.	13.	15.	16.	17.	18.	19.	20.	21.	22.	25.					
		1	1	0	0	1	1	0	0	0	0	0	0					
		–	–	–	–	–	–	–	–	–	–	–	–					
29	290	1	1	2	2	4	3	3	2	1	1	4	1	4	13·8	402	25	526

TABLE III.—*Appearance of Tumours in Forcibly-Bred Progeny of Individual Tumorous and Non-Tumorous (C57 × RIII)F_1 Hybrid Females.*

F1. No. of female with tumour (age of tumour development, days).	F1. No. of female without tumour (age at death, days).	F2. a.	F2. b.	F2. c.	F2. d.	F2. e.	F3. a.	F3. b.	F3. c.	F3. d.	F3. e.	F4. a.	F4. b.	F4. c.	F4. d.	F4. e.	F5. a.	F5. b.	F5. c.	F5. d.	F5. e.	F6. a.	F6. b.	F6. c.	F6. d.	F6. e.	F7. a.	F7. b.	F7. c.	F7. d.	F7. e.
34 (483)	—	—	0/1	—	—	560	280	1/2	50	280	574	226	7/8	88	344	293	203	13/15	87	319	531	226	14/19	74	321	332 (5 alive)	196	23/26	88	310	368 (2 alive)
69 (502)	—	284	2/2	100	285	—	246	3/4	75	282	396	248	4/5	80	328	619	326	8/11	73	398	333	239	12/14	86	330	504	125	23/27	85	279	265
—	21 (748)	—	0/1	—	—	900	403	1/1	100	403	—	216	2/4	50	387	504	304	4/8	50	423	560	303	4/8	50	327	581 (3 alive)	203	8/10	80	301	490 (7 alive)
—	68 (376)	308	1/1	100	308	—	265	4/4	100	314	—	301	6/10	60	331	412	225	12/20	60	348	457	184	20/29	69	289	358 (2 alive)	143	41/50	82	278	348 (5 alive)
—	20 (573)	554	1/2	50	554	719	320	2/4	50	350	514	306	4/6	67	384	389	165	8/11	73	337	476	245	11/22	50	304	421 (1 alive)	260	17/30	57	387	458 (5 alive)
	38 (643)	505	1/2	50	505	520	—	0/4	—	—	587	187	4/8	50	310	571	265	8/14	57	418	536	190	11/25	44	412	520 (4 alive)	206	29/46	63	340	397 (9 alive)

a = Earliest tumour appearance (days).
b = Number of tumours. / Number of mice alive at earliest tumour appearance.
c = Tumour incidence (per cent).
d = Average tumour age (days).
e = Average age at death without tumour (days).

In an attempt to increase, if possible, the mammary tumour incidence in the descendants of (C57 × RIII)F_1 hybrid females, the progeny of these mice was continued for several generations by brother to sister matings and subjected to having as many litters as possible, after saving at least one litter for the next generation. The progeny of two (C57 × RIIIF)$_1$ hybrid females which developed mammary cancer is shown in Fig. 1 and 2. The forcibly-bred progeny of four (C57 × RIII)F_1 hybrid females which had died free from tumours, also continued for six generations, is presented in Fig. 3 to 6. In these figures, the number of mammary tumours which appeared in each successive generation of both the tumorous and non-tumorous hybrids may be seen at a glance as well as the consecutive number of litter to which the mice with tumours belonged, the age at which they developed cancer or died without showing a tumour. It will also be noted that as a rule the hybrid females of the first three generations belonged to later litters than those of the following generations. This was done in an attempt to increase the activity of the mammary tumour agent, should one be present in the hybrid females. It may also be seen in Fig. 3 to 6 that descendants of tumorous females can remain tumour-free for two, three or four successive generations before developing mammary cancer, and that descendants of a non-tumorous female, in spite of reaching tumour age, may show no mammary tumours for as many as five generations before developing breast cancer, in spite of inbreeding and intensive hormonal stimulation. It may be of interest to point out that Andervont (1949*a*, 1949*b*) found the agent to be transmitted through three or four successive generations of susceptible mice without inducing mammary cancer. Further, the study of the distribution of mammary tumours in charts of the progeny of tumorous and non-tumorous hybrids, shown in Fig. 1 to 6, indicates the possibility of the agent being involved in the development of these tumours.

A comparison of the age of the earliest tumour appearance, tumour incidence, average tumour age and average age at death of the progeny of individual tumorous and four tumour-free (C57 × RIII)F_1 hybrid females is shown in Table III. As can be seen, there was little, if any, difference between the forcibly-bred progeny of the two (C57 × RIII)F_1 females which developed breast cancer and the forcibly-bred progeny of the four (C57 × RIII)F_1 hybrid females which died without tumours. Table IV demonstrates the tumour incidence and tumour age in the forcibly-bred progeny of additional nine non-tumorous (C57 × RIII)F_1 females which was continued for three generations. The tumour incidence, although varied in the progeny of the individual hybrids, was lower and the age of breast tumour appearance higher than those in the progeny of the two tumorous and also of the four tumour-free (C57 × RIII)F_1 hybrid females. The progeny of these nine non-tumorous hybrids showed considerable variations in tumour incidence and tumour age; the descendants of two hybrids (No. 27 and 36) showed no tumours in the three generations observed; those of four other hybrids (No. 31, 33, 21 and 72) did not develop tumours until the third generation of inbreeding; the progeny of only one hybrid female (No. 40) developed mammary cancer in the second generation, although all of them had been subjected to considerable hormonal stimulation. In Table V is shown the tumour incidence, the age of appearance of the earliest tumour and the average tumour age in the six generations of the forcibly-bred progeny of the two cancerous (C57 × RIII)F_1 hybrid females. It can be seen that the tumour incidence in all six generations

TABLE IV.—*Appearance of Mammary Tumours in Forcibly-Bred Progeny of Individual Non-Tumorous (C57 × RIII)F_1 Females.*

(C57 × RIII)F_1 No. of female (age at death in days).	Gen. F2.			Gen. F3.			Gen. F4.		
	No. of female.	Tumour age (days).	Age of mice dying without tumours (days).	No. of female.	Tumour age (days).	Age of mice dying without tumours (days).	No. of female.	Tumour age (days).	Age of mice dying without tumours (days).
26 (554)	41 (VI)	—	501	25 (I)	409	—	54 (I)	—	744
							55 (I)	—	767
				27 (I)	—	420	14 (I)	—	744
							15 (I)	—	744
	42 (VI)	—	501	51 (III)	—	636	72 (II)	—	618
				52 (VI)	—	101	30 (I)	278	—
							31 (I)	286	—
	43 (VI)	—	500	65 (III)	—	524	90 (I)	592	—
							91 (I)	512	—
				66 (III)	—	637	12 (II)	571	—
							13 (II)	311	—
31 (582)	20 (IX)	—	559	71 (IX)	—	504	5 (I)	—	556
							6 (I)	—	591
							7 (I)	446	—
33 (665)	48 (II)	—	535	79 (I)	—	556	25 (I)	—	464
							26 (II)	—	480
				80 (I)	—	486	42 (I)	—	634
							43 (II)	526	—
21 (748)	11 (V)	—	832	33 (IV)	—	602	64 (I)	—	689
				34 (IV)	—	674	66 (I)	—	568
							67 (I)	—	227
	13 (V)	—	664	29 (VI)	—	499	60 (II)	—	530
				30 (VI)	—	613	57 (I)	—	571
							58 (II)	—	650
				31*(VI)	—	609	62 (II)	428	—

72 (315)	18 (VI)	—	577	38 (VII)	—	250	20 (I)	—	392
							21 (II)	311	—
				39 (VII)	—	726	23 (VII)	—	453
							24 (I)	—	429
				41 (VII)	—	618	26 (I)	—	409
							27 (I)	—	697
	19 (VI)	—	532	36 (VI)	—	565	17 (I)	702	—
							18 (I)	—	316
40 (397)	38 (V)	—	724	62 (II)	—	659	77 (I)	—	597
							78 (I)	—	515
				63 (II)	—	433	1 (I)	515	—
							2 (I)	—	612
							3 (I)	—	613
	39 (V)	502	—	59 (II)	—	336	87 (I)	—	364
							88 (I)	—	484
				60 (II)	484	—	84 (I)	276	—
							85 (I)	432	—
39 (672)	15 (VI)	—	420	1 (VI)	—	529	1 (I)	—	421
							2 (I)	—	626
	16 (VI)	—	545	73 (XIII)	—	244	39 (II)	391	—
							40 (II)	644	—
				74 (IX)	452	—	15 (II)	—	446
							16 (II)	—	511
36 (606)	8 (IV)	—	582	43 (IX)	—	554	29 (I)	—	341
				44 (IX)	—	448	33 (I)	—	746
							34 (I)	—	750
				45 (IX)	—	662	31 (I)	—	689
27 (478)	24 (V) *	—	491	57 (V)	—	365	74 (I)	—	517
							75 (I)	—	475

Roman numbers in parentheses = number of litter in which the female was born.
* Tumour appeared and regressed.

TABLE V.—*Tumour Incidence in Forcibly-Bred Progeny of Tumorous (C57 × RIII)F_1 Hybrid Females.*

Generation.	Number of mice alive at the earliest tumour appearance.	Earliest tumour appearance (days).	Mice developing tumours. / Mice dying without tumours. Age in months. 4.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.	19.	21.	Number of tumours.	Tumour incidence (%).	Average tumour age (days).	Number mice dead without tumours.	Average age of mice dying without tumours (days).	Number of mice alive.	Average age of mice alive (days).
2	3	284	–	–	–	–	1/0	1/0	–	–	–	–	–	–	–	–	0/1	–	2	67	285	1	560	–	–
3	6	246	–	–	–	1/0	2/0	–	1/0	–	0/1	–	–	–	–	–	0/1	–	4	67	281	2	485	–	–
4	13	226	–	–	–	3/0	1/0	2/1	1/0	–	1/0	–	1/0	1/0	1/0	–	–	0/1	11	85	338	2	456	–	–
5	26	203	–	–	1/1	2/1	3/0	1/0	4/0	4/0	–	1/0	3/0	1/1	–	1/1	0/1	–	21	81	349	5	412	–	–
6	38	223	–	–	–	5/2	3/0	5/0	4/1	3/1	–	4/0	1/1	1/1	0/1	–	–	–	26	68	329	7	381	5	499
7	55	125	1/0	2/1	4/0	5/2	13/1	5/0	3/0	5/0	4/1	1/0	2/0	1/2	–	–	–	–	46	84	292	7	309	2	351

was high, with only slight variations from 67 to 88 per cent. Similarly the average tumour age showed comparatively small changes and was quite low. The majority of tumours in each generation developed during the first 12 months. The age of appearance of the earliest tumour showed gradual decrease from 284 days in the second to 125 days in the seventh generation. In Table VI is presented the age

FIG. 1.—Chart of forcibly-bred progeny of tumorous (C57 × RIII) F_1 No. 69 female.

Roman numbers in parentheses = consecutive number of litter; T = tumour; N = died; L = alive. Number preceding letters T, N or L = number of female; number following letters T, N or L = tumour age or life span in days.

of the earliest tumour appearance, the appearance of mammary tumours according to months, the tumour incidence, and the average tumour age in six generations of combined forcibly-bred progeny of the thirteen non-tumorous (C57 × RIII)F_1 hybrid females. The tumour incidence increased from 19 per cent in the second to nearly 60 per cent in the fifth and later generations. The average tumour age showed a gradual decrease from 16 to 11 months in the seventh generation, and similarly the age of appearance of the earliest mammary tumour

TABLE VI.—*Tumour Incidence in Forcibly-Bred Progeny of Non-Tumorous (C57 × RIII)F_1 Hybrid Females.**

Generation.	Number of mice alive at earliest tumour appearance.	Earliest tumour appearance (days).	Mice developing tumours. Mice dying without tumours. Age in months. 5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.	26.	28.	30.	Number of tumours.	Tumour incidence (%).	Average tumour age (days).	Number of mice dead without tumours.	Average age of mice dying without tumours (days).	Number of mice alive.	Average age of mice alive (days).
2	21	308	–	–	–	–	–	1 / 0	–	–	–	0 / 1	–	0 / 1	2 / 4	1 / 3	0 / 3	–	–	0 / 1	–	0 / 2	–	–	0 / 1	0 / 1	4	19	467	17	592	—	—
3	39	265	–	–	–	–	1 / 0	1 / 0	2 / 1	1 / 1	2 / 0	1 / 2	1 / 2	1 / 3	0 / 3	0 / 3	0 / 2	0 / 3	0 / 3	0 / 3	0 / 1	0 / 2	–	–	–	–	10	26	390	29	553	—	—
4	83	187	–	1 / 0	1 / 1	–	3 / 0	8 / 3	1 / 3	2 / 1	3 / 0	3 / 3	3 / 4	1 / 2	2 / 6	1 / 2	2 / 7	1 / 5	0 / 4	0 / 1	1 / 3	–	0 / 5	0 / 1	–	–	32	30	393	51	512	—	—
5	53	165	–	1 / 0	1 / 0	1 / 0	1 / 2	5 / 0	8 / 0	2 / 1	3 / 2	4 / 3	1 / 1	1 / 1	2 / 2	–	2 / 3	0 / 1	0 / 2	0 / 1	0 / 2	–	–	–	–	–	32	60	372	21	502	—	—
6	94	184	–	3 / 0	0 / 1	6 / 1	10 / 1	6 / 1	5 / 3	5 / 5	3 / 1	2 / 3	1 / 4	1 / 1	2 / 7	1 / 2	0 / 2	0 / 2	1 / 1	0 / 1	0 / 1	–	0 / 1	–	–	–	46	49	325	38	459	10	594
7	166	143	1 / 2	5 / 1	8 / 3	10 / 3	20 / 1	10 / 0	11 / 5	6 / 5	7 / 3	5 / 5	2 / 3	1 / 4	4 / 5	2 / 4	2 / 1	–	1 / 0	–	–	–	–	–	–	–	95	55	318	45	389	26	529

* Progeny of four females continued for six generations, and progeny of nine females continued for three generations.

decreased gradually from 10 months in the second to 5 months in the last generation. The distribution of breast tumours in the normally-bred progeny of (C57 × RIII)F_1 No. 22 female which had also been bred in a normal way is shown in Fig. 7. Again the number of breast tumours which appeared in each successive

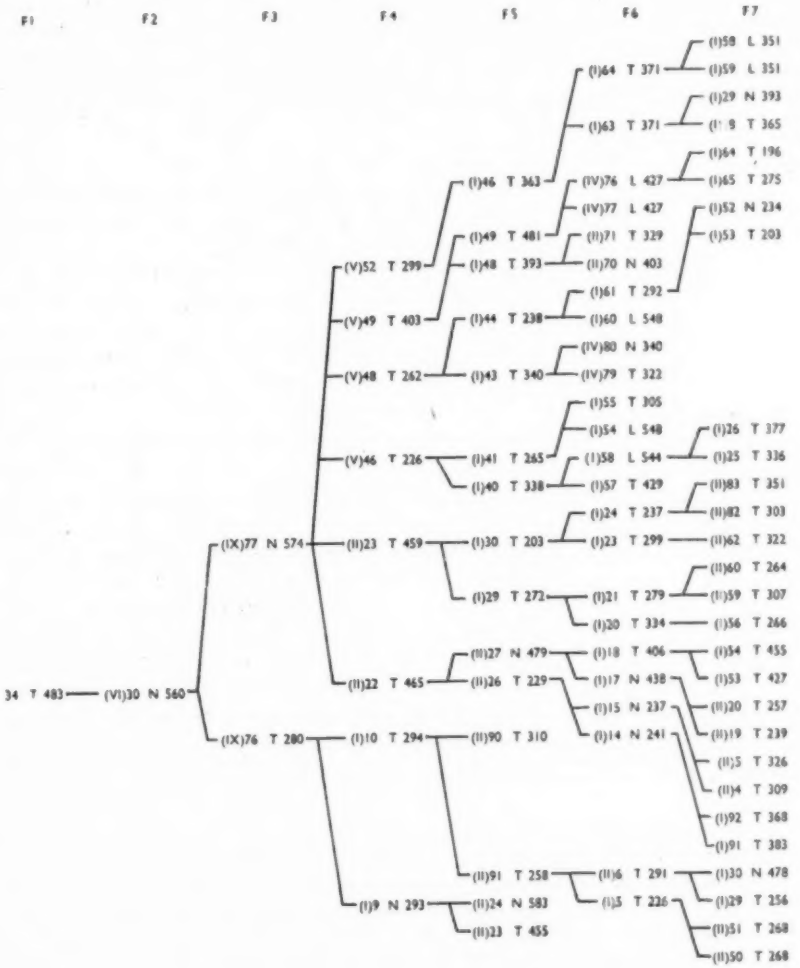

FIG. 2.—Chart of forcibly-bred progeny of tumorous (C57 × RIII)F_1 No. 34 female.

Roman numbers in parentheses = consecutive number of litter; T = tumour; N = died; L = alive. Number preceding letters T, N or L = number of female; number following letters T, N or L = tumour age or life span in days.

generation of the progeny of this non-cancerous hybrid female may be seen in this figure as well as the consecutive number of litter to which the mice belonged which developed tumours. It would have been of interest to obtain the first litter of this female and its progeny and compare any mammary tumour incidence in this progeny with that of the progeny obtained from the sixth litter, but this could not be done owing to limitation of space. Table VII shows the age of earliest tumour appearance, the tumour incidence, and the average tumour age in the normally-bred progeny of this non-tumorous (C57 × RIII)F_1 hybrid female. As can be seen, the age of the earliest tumour appearance decreased gradually from 11 to 4 months in the sixth generation of inbreeding; mice of each generation,

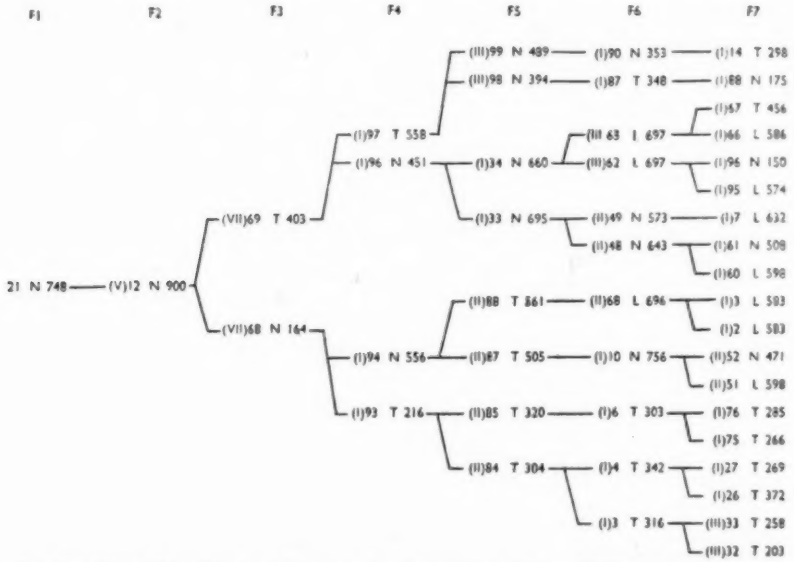

FIG. 3.—Chart of forcibly-bred progeny of non-tumorous (C57 × RIII)F_1 No. 21 female.

Roman numbers in parentheses = consecutive number of litter; T = tumour; N = died; L = alive. Number preceding letters T, N or L = number of female; number following letters T, N or L = tumour age or life span in days.

except the second, developed a high incidence of breast cancer, and the average tumour age decreased from 16 to 11 months in the last generation.

A comparison of the mammary tumour incidence in the progeny of (C57 × RIII)F_1 hybrid females which died free of cancer and in the progeny of (C57 × RIII)F_1 hybrids which developed breast cancer is shown in Table VIII. The breast tumour incidence in the forcibly-bred progeny of the non-cancerous (C57 × RIII)F_1 hybrid females was lower and the average tumour age considerably higher than those in the forcibly-bred progeny of cancerous (C57 × RIII)F_1 hybrid females, but this difference in tumour incidence and average tumour age gradually became smaller in successive generations of the progeny. The difference in the age of the earliest tumour appearance in the descendants of these two types of

TABLE VII.—*Tumour Incidence in Normally-Bred Progeny of a Non-Tumorous (C57 × RIII)F_1 Hybrid Female.*

Generation.	Number of mice alive at earliest tumour appearance.	Earliest tumour appearance (days).	Number of mice developing tumours / Number of mice dying without tumours. Age in months. 4.	9.	10.	11.	12.	13.	14.	15.	16.	17.	19.	20.	21.	Number of tumours.	Tumour incidence (%).	Average tumour age (days).	Number of mice dead without tumour.	Average age of mice dying without tumour (days).	Number of mice alive.
2	1	–	–	–	–	–	–	–	–	0/1	–	–/–	–	–	–	–	–	–	1	447	–
3	4	321	–	–	–	1/0	–	–	–	1/0	–	1/0	–	–	1/0	4	100	481	–	–	–
4	5	424	–	–	–	–	–	–	1/0	1/1	–	1/1	–	–	–	3	60	454	2	477	–
5	13	381	–	–	–	–	–	1/1	3/0	2/1	–	–	0/1	0/1	0/1	6	46	420	5	525	2
6	28	121	1/0	2/1	1/0	3/0	5/1	–	1/0	0/1	0/1	0/1	–	–	–	13	46	325	5	415	10

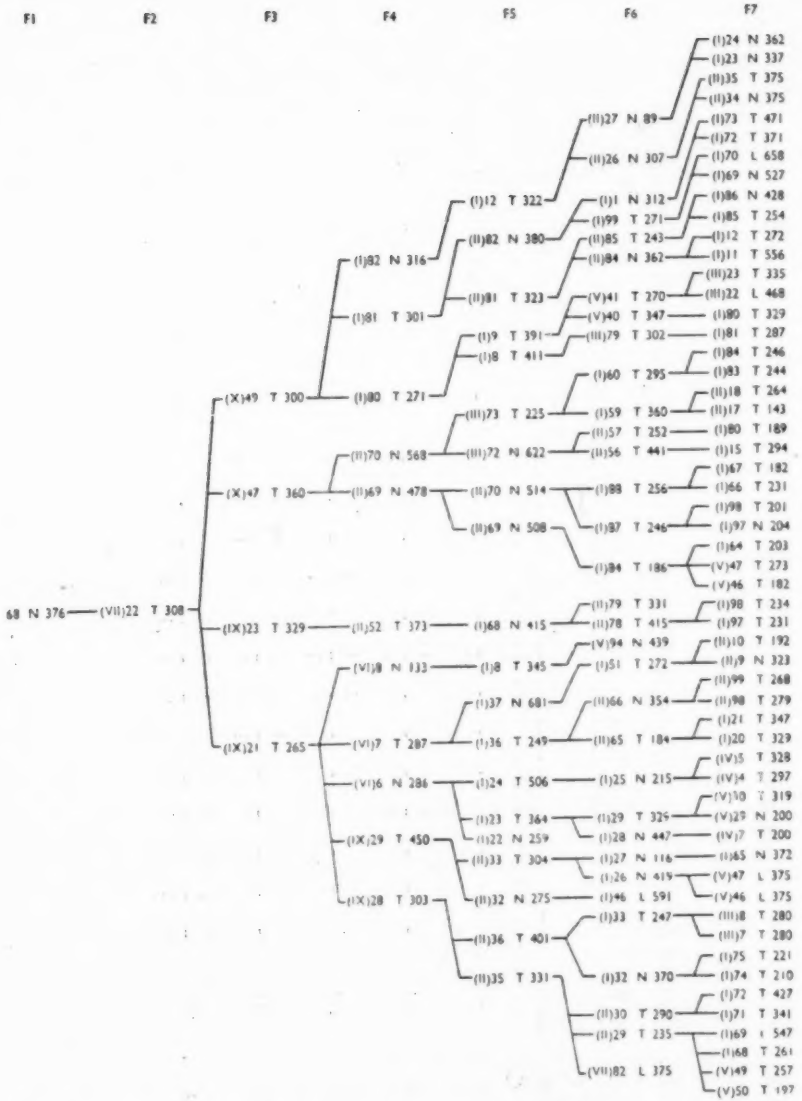

Fig. 4.—Chart of forcibly-bred progeny of non-tumorous (C57 × RIII)F_1 No. 68 female.

Roman numbers in parentheses = consecutive number of litter; T = tumour; N = died; L = alive. Number preceding letters T, N or L = number of female; number following letters T, N or L = tumour age or life span in days.

TABLE VIII.—*Comparison of Tumour Incidence in Progeny of Tumorous and Non-Tumorous (C57 × RIII)F_1 Hybrid Females.*

Generation.	Tumorous females. Forcibly bred progeny.*			Non-tumorous females. Forcibly bred progeny.†			Non-tumorous females. Normally bred progeny.‡		
	Earliest tumour appearance (days).	Tumour incidence (%).	Average tumour age (days).	Earliest tumour appearance (days).	Tumour incidence (%).	Average tumour age (days).	Earliest tumour appearance (days).	Tumour incidence (%).	Average tumour age (days).
F2	284	67	285	308	19	467	—	0	—
F3	246	67	281	265	26	390	321	100	481
F4	226	85	338	187	39	393	424	60	454
F5	203	81	349	165	60	372	381	46	420
F6	223	68	329	184	49	325	121	46	325
F7	125	84	292	143	55	318			

* Progeny of 2 females.
† Progeny of 13 females (of 9 females continued for 3 generations only, and of the remaining females for six generations).
‡ Progeny of 1 female.

hybrid females was less apparent, and in the later generations of the progeny of non-tumorous hybrid females it was even lower than that in the progeny of tumorous (C57 × RIII)F_1 hybrids. It may be of interest to point out that Foulds (1949) recorded a very low breast tumour incidence (1 to 3 per cent) at a late age (16 months) in forcibly-bred progeny of a non-tumorous (C57 × RIII)F_1 hybrid compared with a high (70 per cent) at 11 months in the progeny of three tumorous (C57 × RIII)F_1 hybrid females. The age of the earliest tumour appearance and the average tumour age in the progeny bred in a normal way of one cancer-free (C57 × RIII)F_1 hybrid female was distinctly higher than those in the forcibly-bred progeny of both tumorous and tumour-free (C57 × RIII)F_1 hybrids. The tumour incidence in the normally-bred progeny was, with one exception, lower than that in the forcibly-bred progeny of tumorous hybrid females and, again with one exception, approximated to that in the forcibly-bred progeny of non-tumorous hybrid females. It should be pointed out that the results of the comparison of the behaviour of normally-bred progeny of one non-tumorous female with that of the forcibly-bred progeny of thirteen non-tumorous hybrid females may not be the same as those which would have been obtained should this comparison have been extended to similarly maintained progeny of a number of other cancer-free hybrids which had been bred in a normal way. This point may be seen from the observations on the forcibly-bred progeny of four non-tumorous (C57 × RIII)F_1 hybrids compared with those on similarly treated progeny of two tumorous (C57 × RIII)F_1 hybrid females. The difference in mammary tumour incidence and average tumour age between these two groups of hybrid females became pronounced only after including the observations on the progeny of nine additional non-tumorous (C57 × RIII)F_1 hybrid females. It is therefore possible that the results of the comparison of normally with forcibly-bred progeny of non-cancerous hybrids would have been different as in the case of the comparison of forcibly-bred progeny of non-tumorous with that of tumorous hybrids. It is not known whether the results of Foulds' (1949) comparison of forcibly-bred progeny from tumorous and non-tumorous hybrid females would

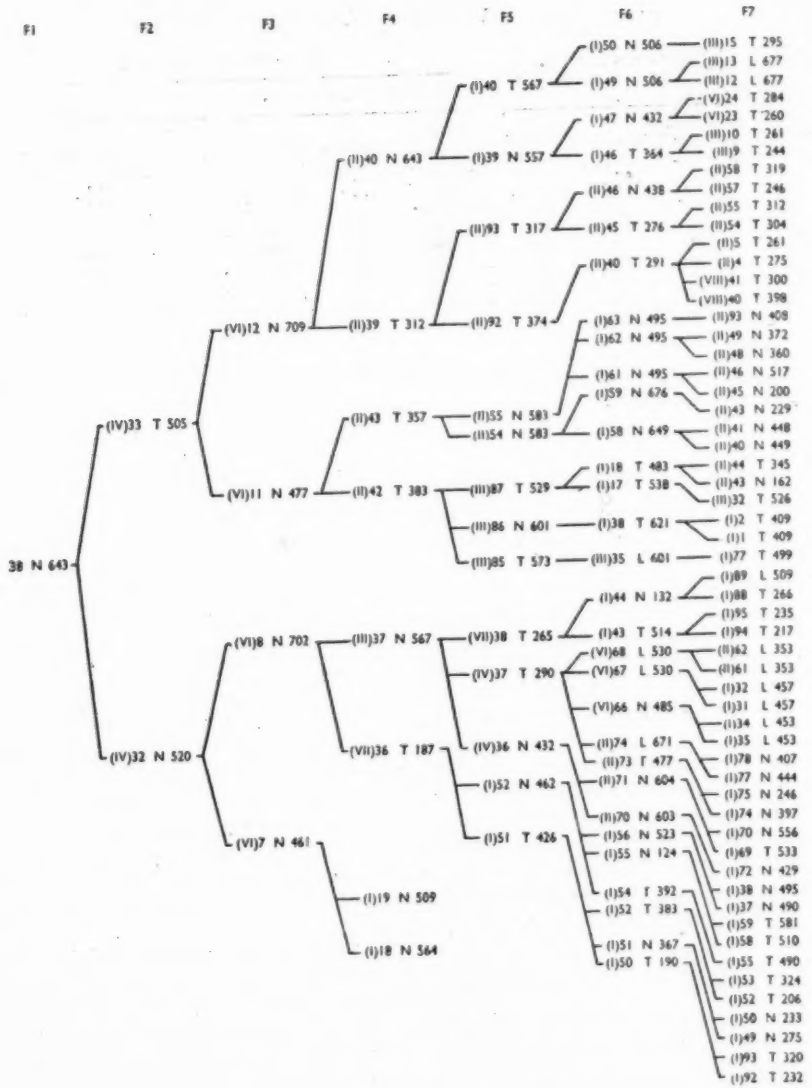

FIG. 5.—Chart of forcibly-bred progeny of non-tumorous (C57 × RIII)F_1 No. 38 female.

Roman numbers in parentheses = consecutive number of litter; T = tumour; N = died; L = alive. Number preceding letters T, N or L = number of female; number following letters T, N or L = tumour age or life span in days.

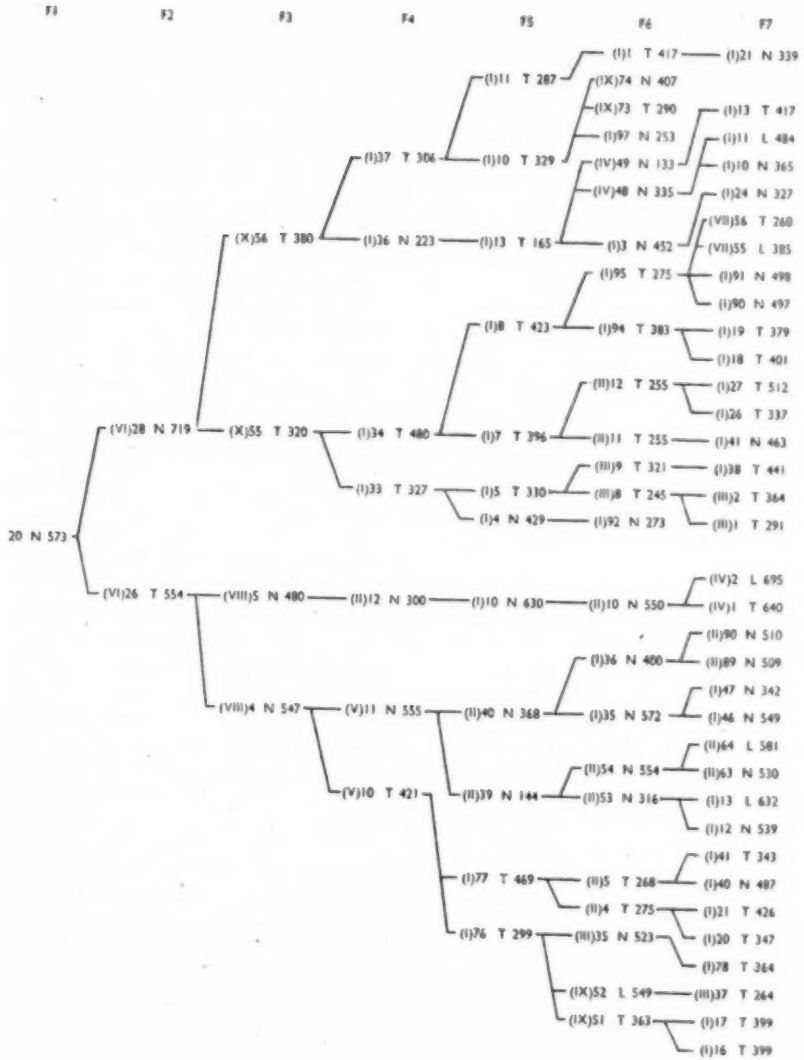

FIG. 6.—Chart of forcibly-bred progeny of non-tumorous (C57 × RIII) F_1 No. 20 female.

Roman numbers in parentheses = consecutive number of litter; T = tumour; N = died; L = alive. Number preceding letters T, N or L = number of female; number following letters T, N or L = tumour age or life span in days.

not have been different had the progeny of a greater number of non-tumorous hybrid females been included in his study.

From the study of the distribution of cancerous females in the forcibly-bred progeny of tumorous and tumour-free (C57 × RIII)F_1 hybrid females and in the normally-bred progeny of a cancer-free hybrid, shown in Fig. 1 to 7 and in Table

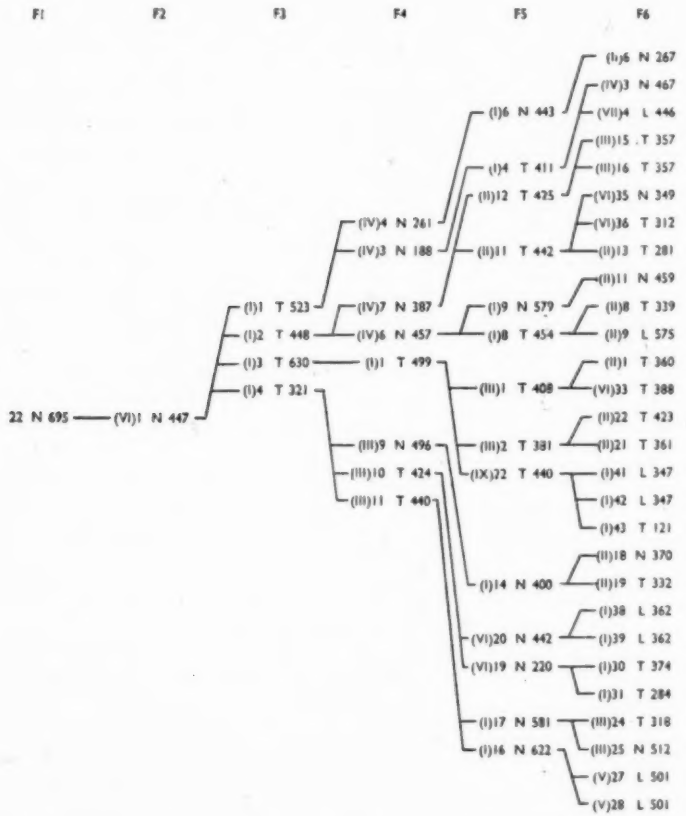

FIG. 7.—Chart of normally-bred progeny of non-tumorous (C57 × RIII)F_1 No. 22 female.

Roman numbers in parentheses = consecutive number of litter; T = tumour; N = died; L = alive. Number preceding letters T, N or L = number of female; number following letters T, N or L = tumour age or life span in days.

IV, conclusions may be drawn about the parent-offspring correlation and the presence of the mammary tumour agent in these lines, especially in females with tumours. Although, perhaps at first sight, the mammary tumours may appear scattered throughout the pedigree-charts of some lines, yet even then in certain lines there appears to take place a segregation of high-cancer lines. Detailed

analysis revealed a more positive parent-offspring correlation. In the progeny of the two (C57 × RIII)F_1 hybrid females with tumours, there were among the descendants of tumour-bearing mothers 82 daughters with tumours and 22 without mammary tumours, and among the progeny of mothers without breast cancer there were 23 daughters with tumours and 4 without breast tumours. In the forcibly-bred progeny of the thirteen cancer-free (C57 × RIII)F_1 hybrid females the tumorous mothers had 134 daughters which developed breast cancer and 67 which died cancer-free, and the non-tumorous mothers had 81 cancerous and 165 non-cancerous daughters. In the normally-bred progeny of one cancer-free (C57 × RIII)F_1 hybrid the segregation was not so evident: mothers with breast cancer had 16 tumorous and 12 non-tumorous daughters, and cancer-free mothers gave birth to 12 cancerous and 12 non-cancerous daughters. Thus in the majority of lines the chance that a female would develop mammary cancer was greater if her mother had a tumour than if her mother died without a breast tumour. In the progeny of the two (C57 × RIII)F_1 tumorous females even the progeny of tumour-free mothers showed a greater tendency towards tumour development than the progeny of tumour-free mothers derived from the thirteen (C57 × RIII)F_1 non-tumorous hybrid females. Therefore, there was a strong indication of the presence of the agent in these hybrids and of its transmission to their progeny which developed mammary cancer either after inbreeding alone or following inbreeding combined with intensive hormonal stimulation.

A search for the possible influence of age on the development of mammary tumours in the hybrid progeny (Tables I, II, V, VI and VII) showed that in all lines the age of tumour-bearing females was distinctly lower than that of tumour-free mice. Thus in the progeny of the two (C57 × RIII)F_1 females with tumours, the tumour-bearing mice lived to an average age of 11 months before the development of breast cancer and the tumour-free mice to an average age of 14 months. In the descendants of the thirteen non-tumorous hybrid females, the average age of mice with cancer was 13 months and of mice without breast tumours 17 months. The average tumour age of mice in the progeny of a non-tumorous (C57 × RIII)F_1 female, which had been bred in a normal way, was 14 months, and the average life-span of mice without breast cancer was 16 months. Thus the age, as such, had no influence on the development of breast cancer in these hybrid females.

As can be seen in Tables IX and X, the study of a possible influence of the number of litters born to each female on the development of breast cancer in the hybrid mice revealed that among the descendants of the two tumorous (C57 × RIII)F_1 hybrid females, the tumour-bearing mice had an average of 6·2 (from 1 to 13) litters and the tumour-free mice an average of 6·7 (2 to 13) litters. Among the forcibly-bred progeny of the four tumour-free (C57 × RIII)F_1 hybrids, tumorous mice had an average of 7·3 (1 to 13) and the mice without tumours an average of 8·3 (1 to 16) litters. A greater number of litters was therefore required before mice developed breast cancer in the progeny of tumour-free (C57 × RIII)F_1 hybrids than that required in the descendants of tumorous (C57 × RIII)F_1 before they showed breast cancer. This difference is even more pronounced in the progeny of nine tumour-free (C57 × RIII)F_1 hybrid females, in which mice with breast tumours had an average of 9·2 (5 to 13) litters and tumour-free mice an average of 9·5 (2 to 16) litters. In the combined descendants of the thirteen tumour-free (C57 × RIII)F_1 hybrids, the average number of litters for tumorous mice was 7·4 (1 to 13) and for tumour-free mice 8·7 (1 to 16) litters. Cancerous

TABLE IX.—*Number of Litters Born in the Progeny of Tumorous (C57 × RIII)F_1 Hybrid Females Before either Tumour Appearance or Death.*

Generation.	No. of litter.													Number of mice alive at earliest tumour appearance.	Number of mice alive.
	0.	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	
1	–	–	–	–	–	–	2/0	–	–	–	–	–	–	–	2	–
2	–	–	–	–	–	–	–	1/0	–	0/1	1/0	–	–	–	3	–
3	–	–	–	–	1/0	1/0	1/0	0/1	0/1	1/0	–	–	–	–	6	–
4	–	–	–	1/0	–	1/1	3/0	2/1	1/0	1/0	1/0	–	–	1/0	13	–
5	1/1	–	1/0	2/2	2/0	4/0	2/0	3/0	1/1	4/0	–	–	1/0	0/1	26	–
6	–	2/0	1/0	0/1	2/0	5/1	3/1	4/1	4/1	2/2	2/0	–	1/0	–	38	5
7	–	–	7/1	4/1	4/1	5/0	6/1	6/0	7/1	4/0	1/2	1/0	1/0	–	55	2

$$\frac{\text{Numerators}}{\text{Denominators}} = \frac{\text{Number of mice developing tumours.}}{\text{Number of mice dying without tumours.}}$$

mice in the progeny of the one (C57 × RIII)F_1 non-tumorous hybrid which had been bred in a normal way had an average of 6·2 (2 to 10) and non-cancerous mice an average of 6·1 (2 to 12) litters. Thus the mice which developed breast cancer in each of the lines did not have more litters than the mice which died without developing a tumour, but the progeny of the thirteen non-tumorous hybrid females had a greater number of litters before the appearance of a mammary tumour than the progeny of the two tumorous hybrids.

A connection between inbreeding and probably hormonal stimulation and the appearance of breast cancer in the progeny of these hybrids was found after an assessment of the number of litters born to females before they developed cancer. As can be seen in Tables IX and X, in mice of each generation of progeny of both cancerous and non-cancerous (C57 × RIII)F_1 hybrids, the number of females which had only five litters before developing a tumour increased in each successive generation, although in all generations the majority of females had more than five litters before the appearance of breast cancer. As shown in Table XI, the number of tumour-bearing females born in the first litter also increased in each successive generation, in spite of the increasing number of tumorous females born in later litters. Further, an increase in the amount of the tumour agent as a result of securing later litters for obtaining progeny for each successive generation and its

TABLE X.—*Number of Litters Born in the Forcibly-Bred Progeny of Non-Tumorous (C57 × RIII)F_1 Hybrid Females Before either Tumour Appearance or Death.**

Generation.	No. of litter.																Number of mice alive at earliest tumour appearance.	Number of mice alive.
	0.	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	
1	–	–	0 – 1	–	–	0 – 1	0 – 8	0 – 1	–	0 – 1	0 – 1	–	–	–	–	–	–	0	–
2	–	–	–	–	–	–	–	0 – 1	0 – 2	2 – 1	0 – 8	2 – 1	0 – 2	0 – 2	–	–	–	21	–
3	–	–	0 – 1	0 – 1	1 – 0	–	1 – 1	2 – 2	2 – 2	0 – 4	2 – 3	0 – 7	0 – 3	3 – 1	–	–	0 – 3	39	–
4	–	–	–	1 – 1	0 – 2	2 – 3	5 – 6	5 – 5	5 – 8	7 – 12	4 – 2	3 – 2	0 – 3	0 – 2	0 – 4	0 – 1	–	83	–
5	–	–	–	–	1 – 1	2 – 2	6 – 2	5 – 0	7 – 5	3 – 1	6 – 6	1 – 2	–	1 – 2	–	–	–	53	–
6	0 – 1	1 – 1	1 – 0	4 – 0	2 – 2	6 – 4	5 – 2	8 – 8	8 – 2	2 – 4	5 – 3	3 – 3	1 – 1	0 – 3	0 – 2	–	0 – 2	94	10
7	4 – 4	1 – 3	0 – 1	5 – 1	10 — 6	9 – 2	14 — 5	8 – 4	17 — 5	10 — 5	5 – 1	8 – 3	2 – 2	1 – 3	–	–	–	166	26

$$\frac{\text{Numerators}}{\text{Denominators}} = \frac{\text{Number of mice developing tumours.}}{\text{Number of mice dying without tumours.}}$$

* Progeny of four females continued for six generations, and progeny of nine females continued for three generations.

TABLE XI.—*Number of Tumorous Mice in the Descendants of (C57 × RIII)F_1 Hybrid Females According to Number of Litter in which they were Born.*

Generation.	Tumorous. No. of litter. 1.	2.	3.	4.	5.	6.	7.	9.	10.	Total.	Non-tumorous.* No. of litter. 1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	Total.
2	–	–	2	–	–	0	–	–	–	2	–	0	–	1	1	1	1	–	0	–	–	4
			0			1				1		1		2	4	8	1		1			17
3	–	–	–	–	–	–	–	1	3	4	1	1	0	0	0	0	1	0	3	4	0	10
								1	1	2	3	3	3	2	1	7	1	2	5	0	2	29
4	2	3	1		5					11	16	11	0		1	1	1	2				32
	2	0	0	–	0	–	–	–	–	2	37	10	1	–	1	1	1	0	–	–	–	51
5	15	4	2							21	17	10	3	1			1					32
	3	2	0	–	–	–	–	–	–	5	9	7	4	1	–	–	0	–	–	–	–	21
6	19	4	3	0						26	24	15	3	0	2	0			2			46
	6	0	0	1	–	–	–	–	–	7	21	12	1	2	1	1	–	–	0	–	–	38
7	26	11	2	2	1		2	2		46	56	14	11	4	5	2	1	2				95
	4	0	0	1	1	–	0	1	–	7	30	14	0	0	1	0	0	0	–	–	–	45

$$\frac{\text{Numerators}}{\text{Denominators}} = \frac{\text{Number of mice developing tumours.}}{\text{Number of mice dying without tumours.}}$$

* Combined progeny of fourteen non-tumorous females.

activation by inbreeding and intensive hormonal stimulation (forced breeding) must also be taken into consideration as a possible cause of the increasing number of tumorous females born in the first litter in each successive generation.

Foulds (1947, 1949) reported an incidence of 15 per cent of mammary tumours at an average age of 10 months in forcibly-bred hybrids of similar derivation. This compares with an incidence of 14 per cent at 13·3 months in (C57 × RIII)F_1 hybrids of the present study. Foulds (1949) noted that the distribution of mammary tumours in the hybrid progeny of C57 Black strain mothers suggested a strong familial factor, the tumours appearing mostly in litters of certain C57 females. This limitation of tumorous hybrid females to certain C57 strain mothers could not be detected in the present study, because although only four hybrid females developed breast cancer, the majority of (C57 × RIII)F_1 hybrids transmitted the tendency to tumour development. Therefore, the progeny not only of tumorous but also the progeny of the majority of non-tumorous (C57 × RIII)F_1 hybrids, in spite of individual variations, developed a high incidence of breast cancer. It appears also that the majority of non-tumorous hybrid females transmitted the mammary tumour agent to their progeny, thus showing no limitation to certain C57 Black strain mothers. It is probable that the majority of mammary tumours are similar to those which developed in the minority of hybrid females or their back-cross progeny in the experiments of Andervont and Dunn (1948*a*, 1948*b*, 1949, 1950*b*). While the tendency to mammary tumour development in the hybrid progeny of susceptible low-cancer-strain females was not limited to certain of these females in the studies of Andervont and Dunn (1950*b*), the tendency to the transmission of the mammary tumour agent was limited to the hybrid progeny of a few females. Thus, the tendency to breast tumour development and/or to transmission of the agent may vary in the hybrid progeny according to the origin of their low-cancer-strain mothers and probably also male parents, and they may also vary in the hybrid progeny of low-cancer-strain mothers from different sublines of the same strain. Foulds (1949) reported data which suggested the presence and transmission of the agent in hybrids of similar derivation to that of hybrids in the present study, but pointed out that his (C57 × RIII)F_1 hybrid females were not bred for determination of mammary tumour incidence. Observations on hybrids of the present experiments strongly indicate the presence and transmission of the agent by at least the majority of hybrid females.

In addition, the incidence of mammary tumours was examined in litters of several hybrid females before and after they had developed tumours. This was done in an attempt at ascertaining any possible increase in the tumour incidence and in the amount of the agent after tumour development (Table XII). In a case of two hybrid females, there was no difference in the number of tumours in mice of litters and several generations of their progeny before and after the development of breast cancer in their hybrid mothers, both types of litters showing a high incidence of mammary tumours. In a case of two other females there were no tumours in litters before they had developed mammary cancer but tumours appeared in litters obtained after these females had shown breast tumours, and this may indicate an increase in the amount of the agent present or its appearance only after the development of a mammary tumour in these hybrid females. The number of tumours increased in the litter of one female obtained after she had developed mammary cancer, and in the case of another hybrid female a tumour developed in a littler obtained before but no tumours in litters secured

TABLE XII.—*Tumour Incidence in Litters Born Before and After the Appearance of a Tumour.*

Generation.	Before tumour development. Number of mice alive at earliest tumour appearance.	(Consecutive no. of litter) Number of tumours.	Average tumour age (days).	Average age of mice dying without tumours (days).	After tumour development. Number of mice alive at earliest tumour appearance.	(Consecutive no. of litter) Number of tumours.	Average tumour age (days).	Average age of mice dying without tumours (days).
				No. 22. *F2 T* (308).*				
		(IX)				(X)		
F3 .	2	2	297	—	2	2	330	—
F4 .	4	4	353	—	5	2	286	454
F5 .	11	7	359	408	9	5	334	506
F6 .	16	8	289	374 (2L)	15	12	289	327
F7 .	26	20	275	298 (3L)	29	21	281	372 (2L)
				No. 21. *F3 T* (265).*				
		(VI)				(IX)		
F4 .	1	1	287	—	2	2	377	—
F5 .	66	4	366	470	3	3	346	—
F6 .	7	3	262	364	7	3	257	395 (2L)
F7 .	11	9	284	262	13	9	275	372 (3L)
				No. 37. *F5 T* (290).*				
		(II)				(VI)		
F6 .	2	1	477	— (1L)	(3)†	—	—	485 (2L)
F7 .	(4)†	—	—	399	(6)†	—	—	— (6L)
				No. 76. *F5 T* (299).*				
		(III)				(IX)		
F6 .	(2)†	—	—	321	2	1	363	— (1L
F7 .	1	1	364	—	3	3	354	—
				No. 13. *F5 T* (165).*				
		(I)				(IV)		
F6 .	(1)†	—	—	452	(2)†	—	—	234
F7 .	(1)†	—	—	327	2	1	417	— (1L)
				No. 10. *F5 T* (329).*				
		(I)				(IX)		
F6 .	(2)†	—	—	218	2	1	290	407
F7 .	—	—	—	—	—	—	—	—

* Tumour age. † All mice included, as no tumours appeared.

after the development of breast cancer. Accordingly it could be deduced that some hybrid females possessed the agent before and after tumour development, which may be the case, as the litters which were compared were late litters; further, some females harboured the agent after tumour development and others showed an increase in its amount, and still others showed the presence of the agent before

and a decrease or absence of it after mammary tumour development. Andervont and Dunn (1949) observed breast tumours in the progeny of a hybrid female before tumour development and no tumours in mice of the litter secured after the female had developed cancer. The behaviour of mice in the progeny obtained after the tumour appeared in their mother may greatly vary even in the case of hybrid females of the same origin, probably as a result of variations in the genetic make-up of individual females influencing the presence or transmission of the agent.

In conclusion it may be stated that both the genetic constitution and hormonal stimulation play an important part in the development of mammary cancer in the hybrid mice which were the subject of the present study, and that the mammary tumour agent also appears to take a part in the origin of these tumours.

Biological Tests for the Presence of the Mammary Tumour Agent.

As soon as the first mammary tumours appeared in the hybrid mice, biological tests for the presence of the agent were carried out. Altogether twenty-three mammary tumours were tested and the results are shown in Table XIII. Of the four mammary tumours which developed in (C57 × RIII)F_1 hybrid females, two showed the presence of the agent and two others failed to reveal the agent under the conditions of the test. The age at which the two negative mammary tumours developed was 10 and 17 months respectively. At first it appears surprising that the tumour which developed at the age of 10 months failed to show the agent, as the time of storage in desiccated state was equal to that of another breast tumour which appeared in the same generation and which, although injected in smaller quantity, induced tumours in the test mice but in distinctly smaller incidence compared with that induced by other dried agent-harbouring tissues in previous experiments (Dmochowski, 1945*a*, 1945*b*, 1946, 1948, 1949*c*). It must be stressed that the method of desiccation of these two as well as of all the other tumours was the same, and the age of the test mice used was in all cases approximately the same. The only varying factors were the amount of tissue injected and the time of storage. The longest period of storage in the ice-chest was that of a negative tumour in a (C57 × RIII)F_1 hybrid female and amounted to 8 months. This should not have made an appreciable difference in the test, as it was previously shown that agent-harbouring tissue induced breast cancer in test mice after two years of storage (Dmochowski, 1946). From the results of the tests of the four tumours in the first generation of hybrid females it may be concluded that two of them possessed the agent, and the two other negative tumours either did not contain the agent or harboured only small quantities or a weak agent which could not be shown under the conditions of the test. None of the tumours of the progeny of (C57 × RIII)F_1 No. 34 female whose tumour was found to harbour the agent was tested, but as can be seen in Fig. 2, from the study of parent-offspring correlation there was a definite tendency towards breast cancer development in daughters of tumorous as well as non-tumorous mothers. From this as well as from the high incidence of tumours it can be presumed that these tumours contained the agent. The results of the tests of breast tumours which developed in the progeny of the tumorous (C57 × RIII)F_1 No. 69 female (Fig. 1), whose tumour failed to reveal the agent, are of interest. Of the nine tumours tested in the progeny of this female, six were found to harbour the agent and three failed

TABLE XIII.—*Tumour Incidence in (C57 × A)F_1 Hybrid Females Injected with Extracts of Dried Tumour Tissue from Various (C57 × RIII) Hybrid Females.*

No. and origin of tumour.	Age at which tumour appeared (days).	Time of storage of dried tumour tissue (days).	Number of injections. Amount of fresh tissue injected (g.).	Number of mice injected.	Number of mice alive at earliest tumour appearance.	Mice developing tumours. Mice dying without tumours. Age in months. 8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.	19.	20.	21.	22.	23.	Number of tumours.	Tumour incidence (per cent).	Average tumour age (months).	Average age of mice dying without tumours (months).
22 F1 (A) (i)	332 (ii)	48	2 / 0·1	18	16	0/1	1/1	–	0/1	1/1	0/2	0/2	0/3	0/3	–	–	–	–	–	–	–	2	13	11	13
67 F1 (A)	290 (ii)	48	5 / 0·25	12	10	–	–	–	–	0/2	0/2	0/3	0/1	–	–	0/1	0/1	–	–	–	–	—	—	—	15
34 F1 (B)	483 (iii)	5	4 / 0·2	6	6	–	–	–	–	1/2	0/2	–	–	1/0	–	–	–	–	–	–	–	2	33	14	13
69 F1 (B)	502 (iii)	238	10 / 0·2	12	8	–	–	–	–	–	–	–	–	–	–	0/3	0/2	–	–	0/3	–	—	—	—	20
10 F2 (B)	284 (T) (iv)	2	4 / 0·2	12	10	–	–	0/1	–	0/1	0/2	0/1	0/1	0/4	–	–	–	–	–	–	–	—	—	—	14
9 F2 (B)	285 (T)	5	4 / 0·1	23	19	–	–	–	0/2	0/3	0/4	0/1	0/4	–	–	0/3	–	–	0/2	–	–	—	—	—	15
22 F2 (A)	308 (N) (iv)	159	8 / 0·2	18	13	0/4	0/2	1/0	0/1	1/1	0/1	0/1	–	–	–	0/1	–	–	–	–	–	2	15	11	11
37 F2 (A)	529 (N)	14	6 / 0·12	14	12	–	–	–	–	–	–	0/1	0/1	–	–	0/1	–	–	–	0/2	–	—	—	—	18
33 F2 (B)	505 (N)	58	7 / 0·175	18	14	–	–	–	0/1	0/5	0/1	–	0/1	0/1	1/0	–	–	0/1	1/1	–	0/1	2	14	19	15
39 F2 (D)	502 (N)	92	4 / 0·1	18	14	–	–	1/0	–	0/1	2/0	0/1	0/1	1/1	1/0	0/1	0/2	0/2	–	–	–	5	36	14	17
15 F3 (B)	276 (T)	14	5 / 0·1	12	10	1/1	–	0/1	1/3	0/1	–	0/1	–	1/0	–	–	–	–	–	–	–	3	30	12	11

| | | |

to show the agent. Mammary tumours of (C57 × RIII)F_2 No. 9 and No. 10, daughters of No. 69 (C57 × RIII)F_1 female, did not reveal the agent, although they developed comparatively early at about 10 months. The (C57 × RIII)F_2 No. 9 female had ten litters and her sister had seven litters. It may well be that the considerable hormonal stimulation was responsible for the appearance of breast cancer in these females, and more likely that the agent took part in the development of these tumours but in quantities too small to be detected by the test. (C57 × RIII)F_3 No. 15 and No. 20, were the two females in the progeny of (C57 × RIII)F_2 No. 9 hybrid whose tumours were tested. The test showed the presence of the agent in the tumour of No. 15 hybrid but not in that of No. 20 female, although the ages at which the two tumours developed were quite comparable. It may well be that had the test animals, in which the latter tumour was tested, lived longer, the agent would probably have been detected. In the fourth generation of the progeny of No. 69 (C57 × RIII)F_1 hybrid, the two breast tumours tested of No. 48 female, granddaughter of No. 9 (C57 × RIII)F_2 female, and of No. 50, daughter of No. 20 (C57 × RIII)F_3 hybrid, were found to harbour the agent. The first tumour developed at the age of 8 months after the female had six litters, and the second at approximately 17 months after the female had thirteen litters. It is of interest that both tumours showed the agent, although the second developed comparatively late in life. In the fifth generation of the same line three tumours were tested, those of No. 89 and 90 females, granddaughters of No. 15 (C57 × RIII)F_3 agent-harbouring female and of No. 48 female, daughter of No. 50 (C57 × RIII)F_4 agent-carrying hybrid. The agent was detected in all three tumours. From the biological tests of mammary cancer in the descendants of the original No. 69 (C57 × RIII)F_1 tumorous hybrid female in whose tumour the agent was not detected, it may be seen how necessary it is to adopt a cautious attitude towards the results of biological tests. It appears probable that the agent may have increased gradually in quantity in successive generations of the descendants of this hybrid female, so that it eventually could be detected by the method applied in the present biological tests. It is possible that the treatment, such as desiccation, may have in part been responsible for the negative results when tumours with small quantities of the agent were tested. Had the mammary tumour of No. 69 (C57 × RIII)F_1 hybrid only been tested and the progeny of this female not raised, the conclusion would have been that increased hormonal stimulation of a susceptible genetic substrate was responsible for the appearance of this breast tumour. It may well be stated that hormonal and genetic factors combined with the mammary tumour agent were responsible for the development of the majority of the tested breast tumours in this line, and probably also a weak or attenuated agent in the tumours negative in the biological test.

In the progeny of non-tumorous (C57 × RIII)F_1 No. 21 (Fig. 3), two tumours, one of (C57 × RIII)F_3 No. 69 and one of (C57 × RIII)F_4 No. 93 hybrids were tested. The agent was found in both tumours. Among the descendants of tumour-free (C57 × RIII)F_1 No. 38 hybrid (Fig. 5) two mammary tumours were tested, one which developed in (C57 × RIII)F_2 No. 33 female at the age of 17 months after she had eleven litters and one which arose in (C57 × RIII)F_4 No. 39 hybrid at the age of 10·5 months after she had given birth to six litters. The agent was detected in both mammary tumours. It is interesting to note the difference in tumour incidence induced in the test mice by these two tumours.

The first, which developed at the age of 17 months, gave only 14 per cent incidence compared with 63 per cent incidence induced by the tumour which developed at the age of 10·5 months. It appears that these two tumours differed in the amounts of the agent present. Among the descendants of the non-tumorous (C57 × RIII)F_1 No. 68 hybrid (Fig. 4) four tumours were examined. The tumour in No. 22 (C57 × RIII)F_2 female developed at the age of 10 months after she had nine litters, and three breast tumours of No. 21, 47, and 49 (C57 × RIII)F_3 hybrids, all of which developed after the eighth litter at the age of 9, 12, and 10 months respectively. All four tumours harboured the agent, yet, although they all arose comparatively early in life, the incidence of tumours induced in the test mice by the tumour of No. 22 F_2 female was only 15 per cent compared with that of 30 to 67 per cent by tumours of the third generation females, and that in spite of the time one of these tumours (No. 49, F_3) was stored, which was three times longer than that of No. 22 F_2 tumour.

An attempt to correlate the appearance of tumours and the presence of the agent with any particular coat colour of the hybrid mice showed that while all F_1 females were of " wild " colour, their progeny in general could be divided into " wild ", " white " and " black " coat colours. Tumours developed in hybrid mice of all three coat colours without particular connection with any coat colour. Foulds (1949) recorded a similar observation in his C57 × RIII hybrids. Neither the presence or absence of the agent, as shown in biological tests, was connected with any particular coat colour.

Foulds (1949) detected the agent in the tumour of a (C57 × RIII)F_1 hybrid which had been tested. Mühlbock (1952) failed to discover the agent in tumours of five (C57 × d) hybrids which developed at ages varying from 20 to 30 months. These hybrids came from early litters. Mammary tumours in hybrids from later litters of C57 females were not tested, but the presence of the agent in these tumours was assumed by Mühlbock (1952) on the basis of a high tumour incidence in the litters, although the average tumour age was 18 months. A search for the agent in mammary tumours of hybrid progeny from another (C) low-cancer-strain but susceptible females and high-cancer-strain (C3H) males led to the detection of the agent in tumours which developed at an early age up to 12 months, although on two occasions even such tumours were negative (Andervont, 1945*b*; Andervont and Dunn, 1950*b*), while mammary tumours arising at a late age, in spite of their high incidence, failed to reveal the agent (Andervont and Dunn, 1949, 1950*b*; Andervont, 1950*a*). Yet, on one occasion a C strain female was found to harbour the agent, although she developed breast cancer at the age of 21 months (Andervont and Dunn, 1950*b*). Thus there may be exceptions in both early and late developing tumours, as shown in the present study with dried tumour tissue and in the experiments of Andervont and Dunn with fresh tumour tissues. Biological tests of some tumours appearing at a late age, as shown in the present experiments, revealed the agent, although they failed in other tumours of similar late age. It is not known how far the recent observation of Andervont (1950*b*) that small quantities of the agent may only be ascertained by observing at least one or even two generations of the descendants of the inoculated test mice would be helpful, but it is certainly worth trying in any future tests of mammary tumours appearing at a late age. In some mammary cancers which developed up to the age of 12 months, the agent could not be detected on several occasions in the present study. Mammary tumour of No. 67 F_1 female at the age of 10 months,

two tumours of No. 9 and 10 F_2 females at the age of 9·5 months and one tumour of No. 20 F_3 hybrid at the age of 8 months all failed to show the presence of the agent. The last three females were descendants of No. 69 F_1 hybrid, whose tumour appeared at 17 months and failed to reveal the agent. All tumours which developed in later generations of No. 69 F_1 hybrid were found to possess the agent. Andervont (1945*b*, 1950*a*) also failed to detect the agent in mice with tumours at 8, 12, 14, 15 and 16 months. On the basis of the study of parent-offspring correlation shown in pedigree charts, it appears probable that the negative tumours also harboured the agent possibly in quantities too small to be detected by the tests applied.

In conclusion it may be stated that the results of the biological tests for the presence of the agent in desiccated mammary tumours which developed in the hybrid mice indicate a gradual accumulation of factors responsible for the appearance of mammary cancer. It appears that there may be a certain threshold below which it is difficult or not possible to show the agent in some mammary tumours, at least by the methods so far employed, and only after combined influence of inbreeding and intensive hormonal stimulation the presence of the agent may become detectable. There is no reason to consider the biological tests as inadequate, in view of the previously reported constant positive results with dried agent-containing tissue, and also in view of the negative results occasionally recorded by other workers with fresh material even from tumours which developed early in life. Further, negative tests for the agent in dried tissue of an 86th transplant of a mammary tumour which originally harboured the agent (Dmochowski, 1952) have been confirmed with fresh tissue of a 101st serial passage of the same tumour. The negative findings may have been due either to small quantities of the agent present in some tumours or to the kind of relationship of the agent to other cell constituents in these tumours, such as close integration with these constituents, different from that in other tumours which were found positive in the biological tests.

Microscopical Appearance of Mammary Tumours.

In view of the availability of a large number of tumours of a comparatively uniform origin a microscopical study of these tumours was made, and also an attempt to correlate the morphological appearance of these tumours with the presence or absence of the agent. Dunn (1945) and Andervont and Dunn (1950*a*) pointed out the difficulties encountered in any classification of mammary tumours, but having a large number of mammary tumours available they made an attempt to classify these tumours into four main types: Type A—with acinar structure predominating; Type B—showing a variable pattern, composed either of solid groups of epithelial cells, or single layers of cuboidal cells having cystic spaces or growing as capillary projections into cysts or cords of cells separated by an abundant connective tissue; Type C—with many epithelial-lined cysts, their epithelial lining invested by a layer of spindle cells; Type D—adeno-acanthoma, with large areas composed of squamous elements (Andervont and Dunn, 1950*b*). Types A and B corresponded with Type I and Types C and D corresponded with Type II of a previous classification (Andervont and Dunn, 1948*a*). The latest classification was adopted in the present study, as it served as a useful basis for a comparison with the findings of the extensive studies of Andervont and Dunn (1950*b*) on mice maintained in the United States.

For comparison with the mammary tumours of C57 × RIII hybrids of the present experiments, a group of 141 mammary tumours which developed in 128 RIII high-cancer-strain mice was available. Of these mice, seven had two tumours and three had three tumours. Seventy-one tumours or 50·4 per cent were of Type A and arose at an average age of 232 days (126 to 430 days); sixty-eight or 48·2 per cent belonged to Type B and developed at an average age of 269 days (145 to 471 days); one tumour (0·7 per cent) was of Type D and was observed at the age of 409 days; one tumour was a carcino-sarcoma and arose at the age of 407 days. As can be seen the majority of tumours in this agent-harbouring strain could be divided into roughly equal numbers of Type A and Type B tumours.

The results of the study of 377 mammary tumours in 327 C57 × RIII hybrid mice are shown in Table XIV. Among the 327 tumorous mice, 32 had two tumours and 9 had three tumours. As shown in Table XIV, the majority of tumours were of Type B. Most of the tumours (70·6 per cent) developed during the first 12 months, and among them 53 were of Type A (19·7 per cent) and 215 (79·9 per cent) were Type B tumours; one unclassified mammary carcinoma also belonged to this group of early tumours. After the first 12 months, 21 tumours were of Type A (19·4 per cent), 77 tumours belonged to Type B (71·4 per cent), 9 tumours were of Type D (8·3 per cent), and 1 tumour was unclassified. An analysis of the distribution of the different types of tumours in the progeny of tumorous and non-tumorous (C57 × RIII)F_1 hybrids revealed that the progeny of both types of females had a number of each type of tumour similar to that found in the total number of all tumours. Among the 120 mammary tumours of the descendants of tumorous hybrid females, there were 19 (15·8 per cent) of Type A, 99 (82·5 per cent) of Type B, and 2 (1·7 per cent) of Type D. Among the 225 tumours in the forcibly-bred progeny of non-tumorous hybrid females, 50 (22·2 per cent) belonged to Type A, 166 (73·8 per cent) to Type B, 7 (3·1 per cent) to Type D, and 2 (0·9 per cent) were unclassified. There were 32 mammary tumours examined in the progeny of a non-tumorous hybrid female which had been bred in a normal way; five (15·6 per cent) were of Type A and 27 (84·4 per cent) belonged to Type B. Among the multiple tumours, two or three types of tumours were frequently encountered in the same female. There was no difference in the distribution of types of tumours in the progeny of hybrids which had been subjected to hormonal stimulation of varying intensity.

No correlation was observed between the type of tumour and its location or size or rate of growth or litter sequence of the tumour-bearing mice or age of the animal at which the tumour developed, except in the case of Type D tumours, all of which developed in mice older than 12 months. Further, there was no correlation between the types of tumours in litter mates or between tumours of mothers and offspring.

Similar observations were reported by Andervont and Dunn (1950*b*) on (C × C3H) susceptible hybrids, in which they found 38 per cent of Type A tumours, 44 per cent of Type B, 9 per cent of Type C, and 8 per cent of Type D. They observed a correlation between the age at which mammary tumours appeared and the type encountered, as in mice of up to 19 months of age 63 per cent were Type A tumours and 33 per cent Type B tumours, and in older mice 31 per cent belonged to Type A and 48 per cent to Type B. In another study (Andervont, 1950*a*) 38 per cent of tumours were of Type A and 46 per cent Type B, and in

TABLE XIV.—*Incidence of Mammary Tumours in C57 × RIII Hybrids According to Histological Appearance.*

Type of tumour.	Age in months.																			Total.	Percentage.
	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.	19.	20.	21.	23.		
A	1	–	1	1	5	14	12	11	8	8	4	3	2	1	2	1	–	–	–	74	19·6
B	1	1	12	13	26	51	38	39	34	20	16	14	8	8	4	4	1	1	1	292	77·5
D	–	–	–	–	–	–	–	–	–	–	2	–	1	5	1	–	–	–	–	9	2·4
Unclassified	–	–	–	–	–	–	–	1	–	–	–	–	1	–	–	–	–	–	–	2	0·5
Total	2	1	13	14	31	65	50	51	42	28	22	17	12	14	7	5	1	1	1	377	100

mice older than 19 months 12 per cent of tumours were Type A and 63 per cent Type B, thus showing a predominance of Type B at all ages. Andervont and Dunn (1950*b*) found Type A tumour more frequently in young mice with the agent and a greater variety of types in older mice apparently without the agent or with a weak agent. They did not detect the agent in a C strain female with a squamous type mammary tumour at a late age, yet in her progeny there appeared a Type A tumour with the agent and one squamous type cancer without the agent. In another two females of the same strain, two tumours of Type A and B respectively both harboured the agent; in their progeny all tumours with the agent were of Type A. The majority of tumours in old mice were similar to those of mice with the agent (Andervont, 1945*b*, Andervont and Dunn, 1949). In hybrid mice obtained from crossings of agent-free low-cancer-strains the majority of mammary tumours were of Types C and D, and in those obtained from one of the parents belonging to agent-free but high-cancer strain the majority of breast tumours were of Types A and B (Andervont and Dunn, 1948*a*). It appears that the genetic constitution of strains used for breeding hybrids influences at least the distribution of the various types of breast cancer.

There was no correlation in C57 × RIII hybrid females between the presence of the agent, as shown in biological tests, and the type of tumour encountered. Of the seventeen tumours in which the agent was found, ten were of Type B, six of Type A, and one of Type D, and among the mammary tumours in which the test failed to reveal the agent, four were Type B and two Type A, cancer (Table XIII). The finding of the agent in Type D mammary tumour which developed at 17 months is of interest, as it appears to indicate that not all squamous type tumours which appear comparatively late need be agent-free tumours, or possibly tumours in which for some as yet unknown reason it is difficult to detect the agent. Two agent-harbouring tumours of (C57 × RIII)F_1 hybrids were of Type A (11 months) and Type B (16 months), and the two other tumours in which the agent was not detected were also of Type A (10 months) and Type B (11 months). Among the fifteen agent-harbouring tumours of C57 × RIII hybrid progeny, five were Type A and arose at the age of 9 to 13 months, nine were Type B and developed at 7 to 17 months, and one Type D at the age of 17 months. Two of Type A, two of Type B, and one Type D tumour developed after the first 12 months. Among the tumours in the hybrid progeny in which the agent was not detected, one was of Type A (18 months) and three of Type B (8 to 10 months). In Andervont and Dunn's experience (1950*b*) the majority of tumours (84 per cent) in hybrids developed at the age of 18 to 29 months and the rest during the first 17 months. They also found no correlation between the microscopical appearance of a tumour and the presence of the agent. Some of the early appearing tumours in which the agent could not be detected were of Type A or B (Andervont, 1945*b*, 1950*a*). Foulds (1949) described the microscopical appearance of breast tumours in his C57 × RIII hybrids as "unremarkable". Mühlbock (1952) found the morphology of tumours in C57 × d hybrids of little assistance in the study of the part played by the agent in the development of these tumours. He also noted more unusual features in tumours presumably free of the agent compared with those harbouring the agent, yet the tumours accumulated in later litters of C57 mice following mating to high-cancer-strain males, in which the agent was presumed to be present, had an appearance like that of agent-harbouring breast tumours, in spite of their late average age

of development (20 months). There appears to be a greater variety in appearance of mammary tumours in mice of strains in which the agent could not be detected (Andervont and Dunn, 1950*a*; 1950*b*; Heston, Deringer, Dunn and Levillain, 1950; Mühlbock, 1952). In the present study all tumours of Type D developed after 12 months of age. Similarly, Andervont and Dunn (1950*b*) found this type of tumour to be very rare in younger hybrid mice in which the agent could be detected, although, as shown in this study, this type of tumour even at a late age may harbour the agent. Therefore the greater frequency of squamous metaplasia in tumours of old hybrid mice, if the age of 17 months could be considered as a comparatively late age, need not indicate at least in every case their development without participation of the agent, contrary to the previous suggestion of Kirschbaum (1949). There is no doubt, however, about the greater frequency of squamous type tumours in agent-free high-cancer-strain or susceptible mice (Gardner, 1947; Heston, 1948; Heston, Deringer, Dunn and Levillain, 1950) than in similar mice with the agent, but they do appear occasionally in agent-carrying strains of mice as observed by Dunn (1945), Andervont and Dunn (1950*b*), and in the present study.

From these observations it may be concluded that no particular microscopical appearance of mammary cancer in hybrid mice can be correlated with the presence or absence of the agent, in spite of the difference in distribution of the particular types in various strains of mice. The distribution of the various types of mammary tumours appears to depend on their genetic constitution derived from the strains used for obtaining the hybrid progeny. This genetic background probably also influences the age at which the different types of tumours develop.

GENERAL DISCUSSION.

Andervont (1945*a*) was first to propose several explanations of the appearance of mammary cancer in hybrid mice, presumably free of the agent. Gardner (1947) observed that mammary tumours in mice deficient of the agent develop late in life, grow slowly and are frequently of the squamous type. Since then a considerable body of data has accumulated, which will be discussed in an attempt to collate all the known observations on breast cancer in hybrid mice with the findings of the present study in order to reveal the respective parts played by the genetic, hormonal factors and the agent in the development of mammary tumours in these mice.

Genetic factors.

The influence of the genetic constitution on the development of breast tumours in hybrid mice was revealed by the variations in the tumour incidence in hybrids obtained from crossings of female and male mice of various low-cancer strains differing in their susceptibility to tumour development in the presence of agent, and also in hybrids from females of these strains mated to agent-carrying or agent-free males of various high-cancer strains. Thus a variable but low tumour incidence was reported by Andervont and Dunn (1948*a*) in hybrids from reciprocal crosses of low-cancer-strain (C, I, C57) mice and a higher but still comparatively low tumour incidence in the hybrid progeny of low-cancer-strain (C) females and agent-free high-cancer-strain (dba-) males, in spite of increased hormonal stimulation. An even higher incidence of tumours developing at a late age (22 months

or even later) was observed in hybrid progeny of susceptible low-cancer-strain (C) females and high-cancer-strain (C3H) males with the agent (Andervont, 1945*b*; Andervont and Dunn, 1948*b*, 1949, 1950*b*). Hybrid progeny of another susceptible agent-free strain (Ax) females and agent-carrying high-cancer-strain (A) males developed no breast cancer, while the progeny of yet another agent-free susceptible strain (C3Hb) females and high-cancer-strain (C3H) males with the agent showed a high incidence of mammary rumours (Bittner, 1952*a*). Hybrid females from low-cancer-strain female mice of low susceptibility to the agent (C57 Black) and agent-harbouring high-cancer-strain (RIII) males developed a comparatively low (15 per cent) incidence of breast cancer, but at a comparatively young (10 and 14 nonths) age (Foulds, 1947, 1949). A similar incidence of tumours in hybrid mice of similar derivation has been observed by the writer, but while in Foulds' experience the distribution of tumours suggested a strong familial factor in their incidence, it was not discernible in the present study. The tumorous and also the majority of non-tumorous hybrids gave rise to progeny with tumours. Although the tumour incidence in the descendants of tumour-free (C57 $\times$ RIII)F_1 hybrids varied, it was comparatively high in the majority of these mice. It appears that the hybrids and their progeny in the present experiments showed both a strong tendency to tumour development and to the transmission of the agent, and neither of the tendencies seemed to be limited to certain C57 Black strain females, in spite of their low susceptibility to the agent (Dmochowski, 1948). Andervont and Dunn (1948*a*) noted a parallelism between the tendency to tumour development and susceptibility to the agent, as most of the tumours which developed in hybrids from various crosses appeared in the hybrid progeny of susceptible, even agent-free, male parents. While the tendency to breast tumour development in the hybrids of Andervont and Dunn (1950*b*) was not confined to some of their low-cancer-strain (C) mothers, the tendency to transmission of the agent was limited to certain of these females.

The influence of genetic factors may not always lead to an increase in the number of tumours, in spite of increased hormonal stimulation. Attempts to increase the tumour incidence in the progeny of hybrids, obtained by back-crossing to agent-carrying males or by brother to sister matings, and subjected to forced breeding, resulted only in a decrease in the tumour incidence (Andervont and Dunn, 1949). Contrary to these findings, in the present experiments, brother to sister matings of descendants of non-tumorous hybrid mice, also subjected to forced breedings, led to an increase in tumour incidence. It appears that the main cause of the difference between these two sets of observations was in the presence of the agent in the progeny of hybrids in the present experiments.

Genetic factors appear also to play a part in the development of breast cancer in low-cancer-strain females following mating to high-cancer-strain males, even in sublines of the same strain. While Andervont and Dunn (1950*b*) reported an incidence of 8 per cent of tumours at a late age in their susceptible low-cancer-strain (C) females, Bittner (1952*a*) observed a high incidence of 59 per cent at 18 months in the same strain females following mating to similar high-cancer-strain (C3H) males. Susceptible females of another strain (Ax) were observed by Bittner (1952*a*) to remain free of tumours after mating to agent-carrying males of two high-cancer-strains (C3H and A), and of another susceptible strain (C3Hb) to develop only a low incidence of breast cancer (3 per cent) when mated to high-cancer-strain (C3H) males. Further the same strain (C) females which

showed a high incidence after mating to one (C3H) high-cancer-strain male developed a much lower incidence (30 per cent) when mated to other (A) high-cancer-strain males. Females of strains with low susceptibility may show similar differences. No tumours developed in C57 Black strain females after mating to males of different agent-carrying strains, A and C3H strains (Bittner, 1952*a*), RIII strain (Foulds, 1949) and in the present study, or d strain (Mühlbock, 1952). In other low-cancer-strain (020 and dz) females an incidence of 8 to 23 per cent was reported by Mühlbock (1952) as a result of mating to either high (d) or low-cancer-strain (020) males. However, at least in some of the observations, differences in the genetic constitution of the males must also be considered as well as possible differences in the agent they harboured.

Thus the genetic constitution plays an important part in the development of mammary tumours in hybrid mice derived from matings of various strains and even in hybrids obtained from different sublines of the same strains.

Hormonal factors.

The influence of intensive hormonal stimulation may also vary in hybrids of different derivations and in their progeny. Forcibly-bred (C57 × RIII)F_1 hybrids developed a 14 per cent incidence of tumours in the present study, while no tumours appeared in their litter mates bred in a normal way. A similar incidence of cancer was also noted by Foulds (1949) in forcibly-bred hybrids of similar origin. Andervont (1950*a*) also reported an increased tumour incidence in hybrid progeny of low-cancer-strain (C) females and agent-harbouring or agent-free high-cancer-strain (C3H) males. In both types of hybrids those with seven or eight litters showed a higher tumour incidence than those with three to five litters. The importance of hormonal stimulation is particularly evident in the origin of breast tumours in hybrid females from mice genetically predisposed to tumour development but lacking the agent (Andervont, 1950*a*). This influence of hormonal factors is not so evident in hybrids from strains with low susceptibility to the agent (Andervont and Dunn, 1948*a*), and also may not always be so apparent in hybrid progeny of agent-free high-cancer-strain males compared with that of progeny from the same but agent-carrying males (Andervont and Dunn, 1949). Although forcibly-bred progeny of both cancerous and non-cancerous hybrids in the present study developed a high, but variable, incidence of tumours, this incidence was not significantly different from that in the progeny of a normally-bred hybrid, except for the age of appearance of breast cancer, possibly because of the limited observation on progeny of only one female.

It is not known how far the consecutive number of litter from which the original hybrids and their progeny originated may have been responsible for the difference between the present observations on high breast-tumour incidence in the descendants maintained by brother to sister matings and the decreasing tumour incidence in similarly maintained progeny hybrids of different derivation (C × C3H) reported by Andervont and Dunn (1949). The hybrids and their descendants came from later litters than those in Andervont's and Dunn's experiments, which originated from the first three litters. Hybrids of similar origin (C × C3H) as well as of other origin (C3Hb × C3H) and their progeny developed a low incidence of breast tumours when derived from the first five litters, and a high incidence, both virgin and breeding, if secured from seventh to tenth litter (Bittner, 1952*a*). An increase of breast tumours in hybrid progeny from later litters of

C57 Black strain females mated to high-cancer-strain (d) males was also noted by Mühlbock (1952). While on one hand these observations may be interpreted as a result of the influence of hormonal factors, on the other hand they have been interpreted (Bittner, 1952*a* ; Mühlbock, 1952) as the result of a gradual increase of the agent during its long latent period, or as an outcome of repeated matings to agent-carrying males leading to the transmission of the agent to females.

Thus increased hormonal stimulation may be responsible for an increase in tumour incidence in hybrids, especially those from agent-free male parents, but is not always separable from that of the tumour agent or of genetic constitution.

The mammary tumour agent.

A consistently lower tumour incidence at higher tumour age was noted in hybrids and their progeny born to males of the same but agent-free strain (Andervont and Dunn, 1948*a*, 1949 ; Foulds, 1949 ; Andervont, 1950*a* ; Bittner, 1952*a*). The incidence varied from 25 to 100 per cent in hybrids from agent-carrying males and between 9 and 50 per cent in those from agent-free males according to the number of litters born to these hybrids (Andervont, 1950*a*), about 4 per cent of the former hybrids developing tumours at an early age, and all of the latter showing only tumours at a late age. It is not known, however, whether the genetic constitution of the agent-free males was not sufficiently different from that of agent-carrying males of the same strain to influence the observed difference in the mammary tumour incidence. Hybrid progeny from females of some (Ax) strains mated to agent-harbouring high-cancer-strain (A) males remained free of breast tumours (Bittner, 1952*a*), as well as that of females of other strains (C57) mated to high-cancer-strain (A) males with the agent (Dmochowski, 1944*b*, 1945*a*). Further, a high incidence of tumours was reported in the progeny of some hybrids derived from matings of susceptible females (C3Hb × Ax) to agent-free high-cancer-strain (C3Hb) males. The breast tumours of these hybrids may at least in part have been produced by genetic and hormonal factors, in spite of the apparent absence of the agent in the male parent. However, the presence of the agent was assumed in these tumours, because of the high incidence of breast cancer in the progeny of the tumorous hybrids (Bittner, 1952*a*). Foulds (1949), Andervont (1950*a*) and Bittner (1950) interpreted the difference in tumour incidence between hybrid progeny of agent-harbouring and agent-free high-cancer-strain males as a result of the transfer of the agent by the male, especially in view of the presence of the agent in various organs of the males (Andervont and Dunn, 1948*a* ; Dmochowski, 1949*c*, Mühlbock, 1950*b*). This does not explain, however, the appearance of tumours in the hybrid progeny of agent-free males and the assumed appearance of the agent in these animals. Further, the difference in the tumour incidence may not always be apparent. Mühlbock, (1952) noted in hybrids from some low-cancer-strain (020, dz) females mated to high-cancer-strain (d) males a similar tumour incidence to that in the hybrids from their litter-mates mated to the same but agent-free (dz) males. These two types of hybrids also induced a similar tumour incidence in susceptible test mice when used as foster mothers. Thus the transfer of the agent by the male could not always be demonstrated. Even after the 10th pregnancy of low-cancer-strain females mated to males with and without the agent, these two types of females when used as foster mothers induced a similar tumour incidence in the test mice. The late

tumour age (19 to 25 months) in both types of hybrids is characteristic and hormonal stimulation was assumed to be the cause of these tumours (Mühlbock, 1952). Thus the influence of the agent present in the male parent may not always be evident, and probably the genetic constitution of the different strains may have been responsible for the different results. Hormonal stimulation may have been another factor responsible for the development of breast tumours in the hybrid progeny of agent-free males. These explanations cannot be put forward for the appearance of breast tumours in hybrids of some low-cancer-strain females mated to high-cancer-strain males without the agent. In these tumours the agent was assumed to be present because of the high incidence of tumours in several successive generations of descendants of these hybrids. The presence of a latent agent or small amounts of it in the " agent-free " males could be taken as another possibility, and an increase of the agent or its activation by crossing of two different genetic constitutions and by increased hormonal stimulation.

The development of mammary tumours in some low-cancer-strain females (Andervont, 1945*b* ; Andervont and Dunn, 1950*b*) and in many females of other low-cancer-strains (Bittner, 1952*a*), the presence of the agent in tumours of these females, in spite of their late (18 months) age of appearance (Bittner, 1952*a*), further the appearance of breast cancer, or increased incidence of tumours in the hybrid progeny of these females from late litters compared with none or low incidence in the progeny of early litters (Bittner, 1952*a*) indicate the transmission of the agent to low-cancer-strain females following repeated matings to high-cancer-strain males harbouring the agent. This conclusion is further strengthened by the failure to find a weak or attenuated agent in females of one susceptible low-cancer-strain, in spite of repeated attempts. Foster nursing of susceptible mice by these females (Andervont, 1945*b*) ; observation of several generations of progeny of mice fostered by these females (Andervont and Dunn, 1948*b*) ; exposure of young females to X radiation (Andervont and Dunn, 1950*b*) oestrogenic stimulation of the females (Andervont, 1950*a*), all failed to reveal the agent. Finally the possibility of an increase of the agent in older females of this strain was not substantiated by the observation of similar tumour incidence in hybrid progeny of both old and young females (Andervont and Dunn, 1949). The development of mammary tumours in the descendants of both tumorous and the majority of non-tumorous hybrid progeny of C57 females which remained tumour-free after mating to agent-carrying males in the present experiments as well as the detection of the agent in the majority of the tumours tested indicate the transfer of the agent by RIII high-cancer-strain males. Although not all (C57 $\times$ RIII)F_1 hybrids developed breast cancer, the transfer of the agent must have taken place in the majority of the females. Similarly, Bittner (1952*a*) noted a high incidence of tumours and the presence of the agent in the descendants of cancerous and cancer-free hybrid progeny of certain strain females mated to agent-carrying males. The presence of the agent was assumed in mammary tumours which appeared in hybrid progeny of later litters of some C57 females mated to high-cancer-strain (d) males (Mühlbock, 1952) because of the high tumour incidence, in spite of the late age at which the tumours appeared. The occasional transfer of the agent to the hybrid progeny in mother's milk was therefore accepted by Mühlbock (1952). In some cases the agent was detected in the tumours of hybrid progeny and their low-cancer-strain mothers, in other cases all attempts failed to reveal it (Andervont and Dunn, 1948*b*, 1950*b*).

Thus in low-cancer-strain females mated to agent-carrying males as well as in their hybrid progeny a variable incidence of cancer was reported, with some tumours revealing the agent in biological tests and some not ; in other tumours the presence of the agent was assumed because of high incidence of breast cancer in the descendants of tumorous and/or non-tumorous hybrid females.

The transfer of the agent by high-cancer-strain males accepted as the most likely source of the origin of the agent present in mammary tumours in hybrid females, there remains the problem of the way in which the agent is transferred to low-cancer-strain females. In view of the failure of Andervont and Dunn (1949) to detect the agent in some low-cancer-strain females after mating to agent-possessing males, they suggested that the females either are not affected by the agent or acquire it in insufficient amounts because of the transfer of the agent in an attenuated form *in utero*. As a result of this transfer, only few tumours in the hybrid progeny develop early and harbour the agent, while the majority of tumours develop late without participation of the agent. Transfer of the agent to embryos, however, appears to be doubtful, because of the absence of the agent in high-cancer-strain embryos (Dmochowski, 1949*c* ; Hummel and Little, 1949). The passage of the agent from embryos, should they become infected *in utero*, is also questionable because of the reported neutralisation of the agent by placenta (Hummel, Little and Eddy, 1949). Transmission of the agent *in utero* would also contradict the basis of the discovery of the agent itself. The increase in tumour incidence in hybrid progeny of later litters (Andervont and Dunn, 1949 ; Bittner, 1952*a* ; Mühlbock, 1952) and the high incidence of tumours observed in the descendants of later litters from hybrid females observed in the present experiments may indicate either a transfer of the agent to females repeatedly mated to agent-carrying males and/or gradual increase in the agent, transferred by mating, during its long latent period under the influence of hormonal stimulation (repeated pregnancies). The low incidence of tumours observed in the hybrid progeny of some derivations need not necessarily be interpreted in the same way as the small number of tumours induced in mature mice which had been given the agent, as suggested by Mühlbock (1952). A high incidence of tumours obtained in mature mice of some strains after repeated injections of material containing the agent (Dmochowski, 1945*a* ; Mühlbock, 1952), and a small incidence in mature mice of other strains (Bittner, 1952*b*), indicate that the genetic constitution is the more likely explanation for the different tumour incidences in hybrids of various derivations. Transmission of the agent by the sperm to low-cancer-strain females, followed by its transmission to their hybrid progeny in the milk of these females (Bittner, 1952*a*), appears to be the most likely way in which the agent gains access to hybrid females, although in females of some strains it may only occasionally take place (Mühlbock, 1952). The observation of several generations of descendants of hybrid females can give a clear picture whether the transfer of the agent has taken place or not, as shown in the present study. Under the same experimental conditions some hybrid females may develop mammary tumours while their litter-mates may fail to show tumours ; their descendants may show a similar variation in their behaviour. Thus, the study of the behaviour of the hybrid progeny of low-cancer-strain females, especially of the descendants of later litters of the hybrid progeny, can only decide whether or not the agent has been transferred by the male parent.

The observations on tumour development and the presence of the agent in

the descendants of the hybrid progeny obtained by mating C57 Black strain females to agent-carrying RIII strain males, made in the present experiments, appear to indicate an interesting possibility in the agent-host relationship which may be the outcome of gradual increase in the amount of the agent or its activation in some hybrid females, while in other hybrid females it appears in quantities large enough to be detected immediately with the development of breast cancer in the first generation of hybrid progeny. This may be on one hand the result of individual variations in the genetic constitution of hybrid females, and on the other hand the outcome of varying quantities of the agent which the hybrid females obtain from their low-cancer-strain mothers mated to high-cancer-strain males. Within the framework of individual differences in the genetic make-up and/or quantities of the agent obtained, inbreeding combined with hormonal stimulation also exerts its influence. Thus we have the situation, encountered in the present experiments, that under the same experimental conditions some hybrid females develop mammary cancer and others (even their litter mates) do not show breast cancer; some of the tumorous females do and others do not reveal the agent; some of the progeny of the cancerous hybrid females may develop mammary tumours which again may or may not reveal the agent, and other progeny of the same cancerous females do not develop tumours; the progeny of non-cancerous hybrid females may develop tumours which contain the agent. The development of breast cancer in some of the (C57 $\times$ RIII)F_1 hybrids only after forced breeding may be explained by the transmission of small quantities of the agent by at least some of the C57 Black strain mothers after mating to RIII agent-carrying males. The high, although varied, tumour incidence in the progeny of these hybrids may be explained by the origin of these females as well as of their progeny from late litters and therefore an increase or activation of the agent in these litters, enhanced by inbreeding and increased hormonal stimulation. Andervont and Dunn (1950*b*) also observed that under the same conditions some low-cancer-strain females developed and others failed to develop breast cancer, and some females with tumours possessed and others lacked the agent. There was also no correlation between the presence of the agent in these females and its presence in their hybrid progeny, cancerous females with or without the agent giving rise to hybrid progeny with only late tumours apparently without the agent, or to progeny with both early tumours with the agent and late tumours without the agent, although they were litter mates. Therefore, again, the presence or absence of the tumour and/or the agent in the mother did not necessarily involve the presence or absence of the agent in her progeny. Andervont and Dunn (1950*b*) noted on several occasions among the hybrid progeny with late tumours that their litter mates had early tumours with the agent, and on one occasion a hybrid female with an early tumour harbouring the agent had progeny with either no tumours or only late tumours without the agent, which suggested the disappearance of the agent (Andervont and Dunn, 1949). Bittner (1952*a*) also reported on the hybrid progeny of some derivations with low mammary tumour incidence that some of them gave rise to descendants with a high incidence of tumours harbouring the agent. Thus the variable results appear to be due to variations in the genetic make-up of both the hybrid females and their progeny, which in turn leads to variations in the amount of the agent transmitted. A more favourable genetic make-up may account for an increased amount of the agent and its detection. or the agent may be present in a constant amount and

its detection is entirely dependent on the genetic constitution and hormonal factors encountered in various individual hybrids.

Transmission of the agent by agent-carrying high-cancer-strain males is further strengthened by the observation of Andervont, Shimkin, and Bryan (1942), which disposes of the possibilty of a contagion of the hybrids or their low cancer-strain mothers and also by the absence of the agent in excreta of mice (Dmochowski and Passey, 1950*a*, 1950*b* ; Mühlbock, 1950*a*). This, of course, does not imply the transmission of the agent to all females, and even those which had obtained the agent and developed tumours need not necessarily transmit it to their progeny. It appears also that the paternal contribution, resulting in different tumour incidences in hybrid progeny of the same low-cancer-strain females and males of different high-cancer-strains, besides being genetic may also be of non-genetic origin, because of the observed differences in the agent present in these various high-cancer-strains (Dmochowski, 1945*b* ; Bittner and Huseby, 1946). Thus, while (C57 × RIII)F_1 hybrids develop breast cancer under certain conditions, no tumours appear in (C57 × A)F_1 hybrid females treated in a similar manner (Dmochowski, 1944*b* ; 1945*a*). Similar observations were made by Bittner (1952*a*) on hybrids of various other derivations. The appearance of mammary tumours in some low-cancer-strain females (C3Hb × Ax) mated to agent-free (C3Hb) males and the high incidence of tumours in their descendants, on the basis of which the presence of the agent was assumed although the tumours were not tested biologically (Bittner, 1952*a*), is one at the moment rather perplexing observation. Sudden "*de novo*" appearance of the agent was therefore also considered as a possible explanation of some of the findings (Andervont and Dunn, 1949 ; Bittner, 1952*a*). This consideration was based on previous observations of Bittner (1941) on the sudden appearance of the agent in some susceptible (Ax) mice, deprived of the agent by foster nursing by low-cancer-strain mothers, which remained free of the agent for seven generations, and on similar observations made recently by Bittner (1952*a*) on susceptible mice of another strain (C3Hb) which remained free of the agent for sixteen generations following foster nursing before the appearance of breast cancer in one of the females, which then gave rise to progeny with a high incidence of mammary tumours. According to the writer's opinion, these observations of a sudden appearance of the agent may only show the length of time during which the agent may lie dormant or the time required before the agent, originally present in small amounts, increases in quantity sufficient to induce breast cancer in combination with other known and unknown factors. Of the known factors, either a change in the genetic constitution of the animal concerned alone and/or increased hormonal stimulus may, at least in part, be responsible for the activation of the agent.

Bittner (1939*a*) reported the appearance of some mammary tumours in mice without the agent, and Strong (1943) stressed the influence of hormonal factors in such tumours. The difficulties encountered in the detection of the agent in breast tumours appearing late in the life of mice and the comparative ease with which it could be shown in tumours developing early (Andervont, 1950*a*), further the isolated tumours in hybrid progeny of early litters and the accumulation of tumours in hybrid mice of late litters (Mühlbock, 1952), led to the conclusion that there may be two types of mammary tumours in mice. One type of tumour would be the result of all three factors taking part in its development, the other type would be the result of hormonal and genetic factors without participation

of the agent (Andervont, 1950*a*; Mühlbock, 1952). However, the possibility of the agent being involved in both types of tumours was not discounted by Andervont (1950*a*). The genetic constitution and intensive hormonal stimulation were considered adequate by Heston, Deringer, Dunn and Levillain (1950) to give rise to mammary tumours in susceptible (C3Hb) mice, originally derived from mice deprived of the agent by foster nursing, but they also stressed that absolute proof of the absence of the agent from such tumours was lacking. The appearance of only a few tumours in the hybrid progeny from reciprocal matings of these (C3Hb) mice with low-cancer-strain (C57) mice with no evidence of an increase in tumour incidence in the hybrid progeny of later litters, as originally reported by Bittner (1944) in mice of agent-carrying strains or in hybrid progeny from low-cancer-strain females and agent-carrying males, again led Heston and Deringer (1952) to suggest that some mammary tumours develop in the absence of the agent. Yet, in hybrid progeny of similar mice of other strains, Bittner (1952*a*) observed the appearance of tumours harbouring the agent. Further, the agent has been detected in some mammary tumours appearing in comparatively old hybrids, as shown by Bittner (1952*a*) and in the present study. In other late tumours as well as occasionally also in early-developing breast cancers the agent could not be detected by any, so far, employed testing procedures as noted by Andervont (1950*a*), Andervont and Dunn (1950*b*), and by the writer.

Thus, intensive hormonal stimulation combined with a suitable genetic background influence the origin of mammary tumours in hybrid mice, increase their incidence, and accelerate their appearance. There is no doubt that the mammary tumour agent takes a part in the development of breast tumours appearing up to a certain age, although not all tumours of early appearance in hybrid mice reveal its presence. The conclusion that tumours in old hybrids do not harbour the agent and are the result of a combined action of hormonal and genetic factors only should at least for the time being be suspended, in view of the discovery of the agent in some mammary tumours appearing at an older age than 15 months and the lack of correlation between any microscopical appearance of breast cancer and the presence or absence of the agent.

SUMMARY.

1. (C57 × RIII)F_1 hybrid females, obtained by mating agent-free C57 Black strain females to agent-harbouring RIII high-cancer-strain males, developed a 14 per cent incidence of mammary tumours at an average age of 13 months after bearing in quick succession an average number of 6·4 litters. Their litter-mates, bred in a normal way, died free of tumours after rearing an average number of 3 litters. None of the C57 strain mothers developed breast cancer, although they lived to an average age of 19 months.

2. The descendants of tumour-free hybrid females which had been subjected to forced breeding, maintained by brother to sister matings for several generations and also forcibly bred, developed a lower incidence of tumours at a considerably higher tumour age than similarly maintained progeny of hybrid females with tumours. The difference between the progeny of these two types of hybrid females gradually became smaller in successive generations of inbreeding. The average tumour age in the normally bred progeny of one tumour-free hybrid which had been bred in a normal way was considerably higher than that in the

forcibly-bred progeny of both tumorous and tumour-free hybrids, and the tumour incidence was lower than that in the descendants of cancerous hybrids but approximated that in the progeny of non-cancerous hybrids.

3. A connection between inbreeding and hormonal stimulation and the development of breast cancer in the descendants of the hybrids was revealed by an increasing number of females which developed tumours after bearing five litters in each successive generation of the progeny of both cancerous and non-cancerous hybrid females. This and the increasing number of mice with tumours born in the first litter may have also been the result of an increase in the amount or activation of the mammary tumour agent.

4. Analysis of parent-offspring correlation in the distribution of tumours in the descendants of the hybrids revealed that the progeny of tumours and of the majority of non-tumorous hybrids had a greater chance to develop cancer if the parent developed cancer than if the parent died without a tumour. Thus there was a strong indication of the presence of the agent in these hybrids and of its transmission to their progeny, which showed an increasing number of tumours following the combined influence of inbreeding and intensive hormonal stimulation. The tendency to tumour development and transmission of the agent shown by the hybrids was not limited to certain C57 strain female parents.

5. The number of tumours which arose in litters and their progeny obtained before their hybrid mothers developed cancer and in those secured after tumour appearance indicated that some hybrids appeared to harbour the agent before and after tumour development, some only after, and others only before the development of breast cancer. This may have been the result of variations in the genetic make-up of individual hybrids and their influence on the presence or transmission of the agent.

6. Biological tests for the presence of the agent in twenty-three mammary tumours were positive in seventeen and negative in the remainder of tumours. The age of tumours in which the agent was detected varied from 7 to 17 months, and that of tumours in which the agent could not be detected from 8 to 18 months. These tests, combined with the observed distribution of tumours in the descendants of hybrid mice, showed that under the same experimental conditions some hybrids developed tumours which either harboured the agent or failed to reveal it, while other hybrids, even their litter mates, died without tumours. Some of the progeny of tumorous hybrids failed to develop tumours and others of the same females developed cancer in which again the agent was either demonstrated or could not be shown. Some of the progeny of tumour-free hybrids developed tumours which harboured the agent. As there was no reason to doubt the adequacy of the tests, it is possible that the results were due to small quantities of the agent or an attenuated agent present in some tumours or to its close integration with tumour cells, or to the agent present in a constant amount in animals with a variable and low but gradually increasing susceptibility. There may be a certain threshold below which it is difficult to detect the agent in some tumours, and it becomes demonstrable in other tumours after inbreeding combined with hormonal stimulation.

7. The study of microscopical appearance of 377 mammary tumours in C57 × RIII hybrid females and of 141 tumours in RIII strain mice showed no correlation between the appearance of a tumour and its location, size, rate of growth, litter sequence of tumour-bearing mice, or their age, except in squamous type tumours.

which as a rule developed in older mice above the age of 12 months. There was also no correlation between the appearance of mammary tumours in parents and their offspring. No connection was found between the appearance of tumours in hybrid mice and the presence or absence of the agent in these tumours. The agent was also demonstrated in a tumour of the adeno-acanthoma type which developed at an age of well over 12 months. The frequency of occurrence of various types of mammary cancer and the age at which they develop appear to be influenced by the genetic constitution of hybrid mice.

8. The implications of these observations in correlation with those recorded on hybrids of other derivations are discussed.

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FURTHER EVIDENCE OF ESSENTIAL DIFFERENCES BETWEEN SOME CHEMICALLY-INDUCED AND VIRUS-INDUCED FOWL TUMOURS.

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WE have previously recorded certain apparently essential differences between chemically-induced transplantable sarcomata in our series GRCH 1–15 and the filterable tumours Rous No. 1 sarcoma, Fujinami myxosarcoma, McIntosh sarcoma No. 5 (Peacock, 1946).

Since our last publication on this subject we have successfully propagated three new induced sarcomata, GRCH 16, 17 and 18. These three tumours occurred in the course of attempts to induce epithelial tumours by injecting into adult White Leghorn fowls a mixture of tissues from 10 to 14-day chick embryos of the same flock, with methylcholanthrene and Sudan IV dissolved in olive oil, following the technique used by Rous and Smith (1945) for inducing epithelial tumours in mammalian embryo tissues. We used a variety of embryonic epithelia, but the only tumours that resulted from these procedures were spindle cell sarcomata of precisely the same familiar pattern as when the carcinogens were inoculated directly into the adult breast muscles. They are made up of fleshy spindle cells arranged in whorls and interlacing bundles, the individual cells being elongated, sometimes with branched ends, with finely granular acidophil cytoplasm, and with an oval nucleus containing generally one small nucleolus. Sometimes the cells are multinucleate, in which case the nuclei are in line one behind another, never in the form of giant cells of the foreign body type. Mitoses occur very frequently, and are often somewhat irregular with bridging and incomplete separation of the chromosomes; but many apparently normal mitoses are also to be seen. Occasionally longitudinal fibrillation of the cytoplasm can be made out, but cross-striation has never been observed in metastases in viscera free from striated muscle such as the liver. The cells have many of the characteristics of muscle cells, but in the absence of cross-striation the term rhabdomyosarcoma does not seem to be justified.

Since September, 1951, we have also propagated the Mill Hill 2 endothelioma. Dessicated MH2 was kindly supplied by Professor Engelbreth Holm, who had preserved the material in the refrigerator from 1939 to 1951. Desiccate reconstituted by grinding in a mortar with 20 volumes of water was injected into 8 White Leghorn chicks from 2 to 8 weeks old. Within 2 to 3 weeks 6 birds developed tumours at the site of injection, and these were histologically identical with the tumour originally described by Begg in 1927.

Subsequently we have maintained this MH2 tumour, and confirmed the infectivity of cell free extracts by direct filtration and by some other methods

that have given positive results with other known filterable tumours, but have been tried unsuccessfully in the past in the case of the chemically-induced tumours GRCH 1–15 (Peacock, 1946). Comparisons were frequently made between MH2 and GRCH 16, and some of these are summarised in Tables I to IV and VII.

Before discussing these experiments a brief account of the 3 new chemically-induced sarcomata is necessary.

TABLE I.—*Infectivity of Filtrates.*

Filter.	GRCH 16.	MH_2
No. 1 Whatman filter-paper 2·1 μ	0/4	—
No. 12 „ „ 1·6 μ	—	2/3
Seitz clarifying pad	0/8	6/10
„ sterilising „	—	0/12
Gradacol membrane 0·7 μ*	—	2/2
„ „ 0·6 μ	0/5	8/8
„ „ 0·045 μ	—	0/3

* Average pore diameter calculated from rate of flow of water.

Numerators give number of birds that grew tumours.
Denominators „ „ tested.

TABLE II.—*Ascending Paper Chromatography.*

Height above fluid surface (cm.).	GRCH 16.	MH_2.
2	0/4	2/2
3	0/4	2/2
4	—	1/2
5	—	2/2

No. 1 Whatman filter-paper strips 1 cm. wide with lower edge just touching fluid surface of tumour homogenate.

Numerators give number of birds that grew tumours.
Denominators „ „ tested.

GRCH 16.

20.xii.48: (*a*) Liver and (*b*) squamous epithelium from the crop of a 10-day-old chick embryo were mixed with 1 per cent methylcholanthrene dissolved in a saturated solution of Sudan IV in olive oil. One ml. of mixture (*a*) was injected into the right and 1 ml. of mixture (*b*) into the left pectoral muscles of two 122-day-old White Leghorn pullets, No. 3085 and 3086.

29.ix.49: No. 3086 was killed in moribund condition. There was an oval tumour 5 cm. in greatest diameter in the left pectoral muscles. There was no tumour in the right pectoral muscles, and no residual fluorescence was detected at either site of injection. There was a chronic plastic peritonitis, probably from a healed perforation of the gut, which was bound down with adhesions. The gall bladder was greatly distended. The ovary was inactive. There were no metastases and no other abnormality was seen.

The tumour was firm and white; the cut surface showed a whorled pattern. Histologically the tumour is an undifferentiated spindle cell sarcoma (Fig. 1). About 1 g. of fresh tumour was ground smooth in a sterile mortar, and 10 ml. of 5 per cent dextrose saline were added and well stirred to give a turbid cell suspension.

Ten 6-week-old White Leghorn chicks were inoculated with doses of 0·2 ml. to 1·0 ml. in the right pectoral muscles. Two died early in the experiment, and

the remaining 8 birds all developed palpable tumours at the site of injection within 6 to 12 weeks.

Thereafter the tumour has been maintained by serial passage, and in November, 1952, was in the 17th serial generation of transplantation.

GRCH 17.

11.x.49: No. 3085 was killed. There were large tumours in both breast muscles, spots of violet fluorescence on both sides, and metastases in pancreas, kidneys and lungs.

Histologically this tumour was a pleomorphic sarcoma in which many cells resemble muscle cells (Fig. 2).

This tumour was maintained through 4 serial passages by injection of cell suspensions but was not transmissible by cell free filtrates. The grafted tumours conformed to the general appearance of the original tumour.

GRCH 18.

22.xii.48: White Leghorn Hen No. 3119, 857 days old, was injected in the right pectoral muscles with 10-day chick embryo skin and in the left pectoral muscles with brain from the same embryo; both tissues were mixed with 1 per cent methylcholanthrene solution in saturated solution of Sudan IV in olive oil.

30.iii.49: Of 1 per cent solution of methylcholanthrene in benzene 0·2 ml. was injected into the site of the previous injection in the right breast.

7.xii.49: A tumour was palpable in the right breast. The right wing web was punctured with a thermocautery.

12.i.50: The right wing web was punctured with thermocautery at a second site.

6.iii.50: Enormous tumours were present in both breasts, having spread from right to left side. The bird was killed. No fluorescence was detected at sites of injection nor in the tumours. There were no tumours at sites of cauterisation and no metastases. The ovary was inactive. No other gross abnormality was seen.

Histologically the tumour was a pleomorphic sarcoma. A 1 in 10 fresh cell suspension in 5 per cent dextrose saline was injected into the breast muscles of 6 White Leghorns, 9 to 12 months old, in 2 of which tumours were palpable in about 4 weeks. Subsequently the tumour was maintained through 3 serial passages, by cell suspension inoculation. The histological features of the tumour strain remained unchanged.

As in previous experiments, we have employed a variety of techniques to test for the possible presence of viruses in these induced tumours (Table VII).

Filtration.

Rous No. 1 sarcoma virus is well known to pass through gradacol membranes of average pore diameter (= a.p.d.) 0·6μ–0·7μ. MH2 virus also passes these membranes. Both these tumours were also successfully transmitted by filtration through Whatman filter-paper of a.p.d. 1·6μ–2·1μ and through Seitz clarifying pads.

GRCH 16, 17 and 18 tumours yielded negative results with all types of filtration tested (Tables I and VII).

Ascending Paper Chromatography.

An equally reliable and much simpler method than filtration through bacteria-proof membranes is the use of ascending paper chromatography employed, though not under that name, by Bedson and Bland in 1929 for the isolation of psittacosis virus. We have frequently used this simple technique for the isolation of the infective agents of the filterable fowl sarcomata and found it equally satisfactory for separating the MH2 agent from cell suspensions of this tumour.

By adding a loopful of a culture of *Bacillus prodigiosus* to tumour homogenate suspensions before starting chromatography, and by plating out the paper on nutrient agar at the end of the experiment, it is possible to show that the bacilli do not ascend to the highest level to which the fluid rises.

Experiment.

A parallel test of GRCH 16 and MH2 tumours was carried out as follows:

Of each tumour 1 g. was homogenised in 10 ml. sterile normal saline and one loopful of *B. prodigiosus* was added. One ml. aliquots of each tumour suspension were transferred to sterile tubes, and a 6 cm. × 1 cm. strip of No. 1 Whatman filter-paper marked in half centimetres (sterilised at 140° C. for 20 minutes) was suspended in each tube so that the fluid meniscus was just touching the bottom of the paper strip. The fluid ascended to the 4 cm. mark in 10 minutes. Sample papers from each tumour extract were used for culture. The lowest 1 cm. was cut off and discarded, and the remainder of the paper was laid on a sterile agar plate and flooded with melted agar at 46° C. A control chromatogram of *B. prodigiosus* culture, similarly diluted in saline, and a control sterile paper were also plated (Fig. 3).

Though fluid rose to 4 cm., *B. prodigiosus* was present only in the lower 3 cm. of the wet strip. All levels of wet paper, however, were infective in the case of the MH2 tumour, but in the case of the GRCH 16 tumour no level above the meniscus of the tumour extract was infective.

As in the case of earlier chemically induced sarcomata in which the test was made by similar technique, using paper at levels above the first centimetre from the free fluid, GRCH 16 and 17 homogenates yielded only negative results. This observation is completely in keeping with the results of the more conventional filtrations through gradacol membranes or Seitz clarifying pads. The simplest explanation of the non-infectivity of filtrates of the GRCH tumours is that they contain no specific infective virus. On the other hand, the tumour itself might be supposed to contain such an agent, which for some reason or other was adsorbed strongly by the filter.

In the case of paper chromatography the whole paper can be injected either intact or homogenised in saline, and this might be thought to favour the demonstration of viruses where filtrates yield negative results. The intense foreign body giant cell and fibroblast reaction around the cellulose fibres provides susceptible cells for infection by either the Rous Sarcoma No. 1 or MH2 viruses (Fig. 4).

Action of Ultra-violet Rays.

Tumour cells if well dispersed in saline to give barely cloudy suspensions and if stirred by a gentle stream of air can readily be killed by exposure to ultra-violet rays.

Using the technique of Baker and Peacock (1926), we irradiated simultaneously suspensions of MH2 and GRCH 16 tumours symmetrically placed beneath a high pressure mercury arc. As there are many variable factors in such exposures, it is convenient to express dosage as time related to sterilising effect on bacteria.

Under our conditions suspensions of *B. prodigiosus* were killed in under 2 minutes as tested by subsequent plating on nutrient agar and incubation at 37° C.

The irradiated tumour suspensions were injected after the different lengths of exposure into young White Leghorn chicks from the same hatch which were observed for 3 months before being recorded as negative.

In the second experiment MH2 tumour suspension was exposed for longer periods and after 55 minutes was still infective (Table III).

TABLE III.—*Action of Ultra-violet Rays.*

Duration of exposure (minutes).	GRCH 16.	MH 2.
0	3/3	3/3
12	0/2	1/1
21	0/1	1/1
35	0/1	—
55	—	1/1

Numerators give number of birds that grew tumours.
Denominators „ „ tested.

Action of X-rays.

Two shallow cells in a piece of Perspex were used for the simultaneous irradiation of homogenates of MH2 and GRCH 16 tumours. After each exposure the suspensions were injected into young White Leghorn chicks from the same hatch. The result was only considered negative after a period of 3 months' observation (Table IV).

TABLE IV.—*Action of X-Rays.*

Dosage.	GRCH 16.	MH2.
10,000 r	0/1	1/1
20,000 r	0/1	1/1
40,000 r	0/1	1/1

Numerators give number of birds that grew tumours.
Denominators „ „ tested.

Action of Ultrasonic Waves.

It seemed possible that ultrasonic shaking might destroy the infectivity of tumour viruses, or of cells, or both within a short time. Tumour homogenates freed from gross clumps by preliminary sedimentation at 2000 g. for 5 to 10 minutes were exposed in plastic tubes to a focused beam of ultrasonic waves with a frequency of one megacycle per second transmitted through a cooling water bath. The plastic tube was transparent to the waves, and was so placed that maximum disturbance occurred in the centre of the tube.

In order to concentrate the effect of the waves as much as possible 2 to 5 ml. of 1/10 homogenate of fresh tumour in normal saline were used. The fluid was in vigorous agitation throughout the exposure, and it was assumed that the energy

absorbed was evenly distributed throughout the volume exposed. At the end of exposure the temperature in the tube was 27° C.

GRCH 16, MH2 and Rous No. 1 tumour homogenates so exposed yielded positive results on injection into young White Leghorn chicks.

Examination by phase microscopy of the exposed homogenates revealed many intact nuclei in all cases, though the cytoplasm was difficult to detect, and there was an increase of amorphus debris.

Localising Factors.

The localising effect of proliferative reactions was observed in 1912 by Rous, Murphy and Tytler at the site of injections of diatomaceous earth and at other foci of proliferation. In 1916 Pentimalli showed that cauterisation could localise the virus of Rous Sarcoma No. 1 in the resulting proliferative reaction tissue.

We have previously reported the localisation of virus-induced fowl sarcomata at remote sites of X-ray or radium burns (Peacock, 1946). Cauterisation with a red hot platinum wire or with fuming nitric acid applied to the skin with a glass rod (2 to 3 mm. diameter) has more recently been found to localise the virus in birds bearing Rous Sarcoma No. 1, or less certainly MH2 endothelioma. In our experience, localisation has only been successful in birds already bearing tumours (Fig. 5 and 6). The following experiment illustrates this type of localisation.

Experiment.

1.vi.50 : Ten White Leghorn chicks, 6 weeks old, had a cotton thread (Coats No. 40) impregnated with mucoid fluid from a fresh Rous Sarcoma No. 1 inserted in the pectoral muscles. This procedure is a convenient way of localising for a time the tumour at the site of insertion.

6.vi.50 : Nine chicks had early tumours in the right breast. A sterile cotton thread was inserted in the left pectoral muscles of each chick and also (*a*) in 5 the left wing was injected with 0·1 ml. of a saturated solution of Sudan IV in tricaprylin ; (*b*) in the remaining 5 chicks nitric acid was momentarily applied with a 2 mm. glass rod to the web of the left wing.

20.vi.50 : (*a*) Four out of 5 chicks had tumours in the right breast but no localisation in the wing. (*b*) All 5 chicks had tumours in the right breast. Two chicks had tumours at the cauterised site in the left wing, and one also had a tumour at the site of the thread in the left breast. In the remaining 3 chicks the left wing webs were again cauterised in the same way.

23.vi.50 : The 9 chicks with large tumours at the site of Rous sarcoma inoculation were killed. Three chicks out of 6 had localisation at site of cautery, and 1 of them had localisation at site of control thread.

None of the remaining 3 tumour-bearing chicks had localisation either at the site of Sudan IV injection or at the site of the control thread. One survived till 24.viii.51 without tumours, though re-injected with Rous desiccate on 9.ii.51 and injected with 100-hour embryo + metholcholanthrene on 14.ii.51.

Before, or at the time of intravenous injection of infective filtrates cauterisation has failed to localise the tumour, but some birds grew tumours at the site of veni-puncture, and cautery then applied at a remote site caused localisation of typical Rous sarcoma.

Analysis of the results shows that localisation at the site of a single cauterisation or acid burn occurred only in birds, bearing tumours at the site of injection, and cauterised within a period of 3 to 8 days after injection (Tables V and VI).

TABLE V.—*Results of Attempts at Remote Localisation in Tumour-bearing Birds.*

	Cautery.	HNO_3.	Paper pulp.	Thread.	Sudan IV.	Histamine.	Feather plucking.	Accidental.
GRCH 16	0/14	0/7	0/5					
GRCH 17	0/8	0/3	—	0/3	0/1			
Rous	10/10	6/16	0/5	1/10	0/5	0/5	0/12	2 wing tab trauma.
MH 2	1/9	—	—	—	—	—	—	1 wing tab trauma.

Numerators give number of birds that grew tumours.
Denominators „ „ tested.

TABLE VI.—*Localisation Related to Time of Injection and Growth of Tumour.*

Tumour.	Interval between tumour injection and trauma (days).	Palpable tumour at site of injection before or after trauma.	Tumour incidence at site of— Cautery.	Nitric acid.
Rous	3	After	1/3	
„	4	„	0/1	
„	5	Before	—	3/5
„	6	„	3/3	
MH2	6	„	1/2	
Rous	7	„	0/6	
„	8	„	—	2/3
MH2	14	„	0/3	
GRCH 17	14	12 days after	0/2	
Rous	22	Before	0/1	0/1
„	27	„	9/1	9/1
GRCH 16	32	4 and 8 days before	0/2	0/2
Rous	33	Before	0/1	0/1
GRCH 17	34	1 day before	0/2	
MH2	36	Before	0/2	
„	42	„	0/2	
GRCH 16	42	3 days before	0/1	
„	44	9 „ „	0/3	
„	47	12 „ „	0/3	
GRCH 17	55	18, 23, 31 days before	0/3	
GRCH 16	65	14, 18, 25, 25 days before	0·4	

Numerators give number of birds that grew tumours.
Denominators „ „ tested.
All positive results are included above the dotted line.

Early GRCH tumours growing from cell suspensions are more easily palpable than early virus induced tumours ; no attempt has been made to give the exact duration of tumour growth for the latter.

Though on a very small scale, the results suggest that in birds bearing rapidly growing Rous sarcomata there may be a period of active viraemia, during which localisation at the site of cautery is demonstrable.

We hoped by cutting serial sections of cauterized sites at various time intervals after the injury to recognise the mechanism of localisation, but no specific cytological reaction has been identified as peculiarly favourable to the fixation of the virus. It seems however that the presence of foreign body giant cells, and possibly of necrotic tissue, is in some way essential to the mechanism, as less drastic trauma such as is caused by the insertion of a sterile thread has only

once localised the tumour, though this procedure stimulates a great proliferation of foreign body giant cells. On the other hand, occasionally local inflammatory reaction occurs around the metal wing tabs used for numbering the birds, and we have seen 2 cases of localisation of Rous Sarcoma No. 1 and 1 of MH2 endothelioma in such chronically inflamed sites (Fig. 7 and 8).

Slight trauma involving subcutaneous haemorrhage is caused by plucking the wing feathers. Twelve 4-month-old White Leghorn fowls were injected with cell suspension of Rous Sarcoma No. 1, and had 1 feather plucked from the leading edge of the wing on the day of injection and on the 4th, 6th, 13th and 15th days thereafter. No localisation occurred at the sites of trauma. It was thought that histamine-like action following cautery might cause local blood stasis and so favour the metastatic growth of blood-borne emboli from the tumour at the damaged site. Histamine was therefore injected into the left wing web of 6 White Leghorn chicks 4 weeks old bearing Rous Sarcoma No. 1 injected previously in the right breast. No localisation occurred in these birds within 6 days, by which time the birds were moribund.

Various attempts to localise Rous Sarcoma No. 1, MH2 endothelioma and GRCH 16 and 17 sarcomata are summarised in Table V.

Cross Immunity.

At the second serial passage of GRCH 17 sarcoma a cell suspension was injected into the right pectoral muscles of White Leghorn Hen No. 3285, which had borne a retrogressing Rous Sarcoma No. 1 in the wing, and in which attempts to localise the virus of that tumour by repeated cautery puncture of the skin and muscle of the left breast had failed.

Experiment.

3.xi.49*:* On the 3rd, 4th, 5th, 7th and 9th November successive cautery punctures were made through the skin and underlying pectoral muscles of the right breast of White Leghorn Fowl No. 3285, starting at the upper sternal level and leaving about 1 cm. of normal skin between punctures.

10.xi.49*:* Of Seitz clarified filtrate of a suspension of homogenised frozen Rous Sarcoma No. 1 that had been kept for 56 days at — 20° C., 0·5 ml. was injected into the right alar vein.

11.xi.49*:* Successive cautery punctures were made through the skin and underlying muscles of the left breast as described above on 11th, 14th, 15th, 16th, 17th, 18th and 21st November.

28.xi.49*:* A tumour was palpable at the site of intravenous injection in the right wing.

13.xii.49*:* The wing tumour was retrogressing. There was no localisation of tumour at any cauterised site.

23.ii.50*:* Of cell suspension of Rous Sarcoma No. 1 from the same source as the above infective filtrate, but kept for 105 days at — 20° C., 1 ml. was injected into the left pectoral muscles and 1 ml. of fresh cell suspension of GRCH 17/1 (No. 3281) was injected into the right pectoral muscles.

28.iv.50*:* A large tumour was present in the right breast and the bird was killed. There was direct spread from the GRCH 17 tumour injection site to the liver, but no tumour at the site of Rous No. 1 injection nor at any of the cautery

TABLE VII.—*Comparison between All Tests for Virus in GRCH 16, 17, 18 Sarcomata and Representative Positive Tests in Rous No. 1 Sarcoma and MH2 Endothelioma*

Tumour strain.	Cell suspensions.	High speed mincer.	Filtrates through— Whatman paper a.p.d.* 1·5–2.	Seitz clarifying pads.	Gradacol membrane a.p.d. 0·6–0·7.	Ascending paper chromatography.	−20° C.	U.V.	X ray	Ultrasonic.
GRCH 16	94/113	0/16	0/4	0/20	0/9	0·5–3 cm. 0/11	0/3	0/4†	0/3†	7/18
„ 17	14/23	—	—	0/8	—	1–3 cm. 0/4				
„ 18	4/11	—	—	0/4	—	—	0/5			
MH2	30/30	2/3	2/3	6/10	10/10	2–5 cm. 7/7	—	7/7	3/3	
Rous No. 1	Transmissible by all above methods					2 cm. 4/4	3/3	—	—	2/2

* a.p.d. = average pore diameter in microns.
† See Tables III and IV.

Numerators give number of birds that grew tumours.
Denominators „ „ tested.

sites. Histologically the tumours in the breast and liver were typical GRCH 17 sarcoma (Fig. 9). A fresh cell suspension in dextrose saline of this tumour was prepared, centrifuged at 2000 g. to precipitate gross particles, and 0·5 ml. injected into 21-day-old White Leghorn Chick No. 3324. A cell suspension was filtered through a Seitz clarifying pad and the filtrate injected into 25-day-old White Leghorn Chicks No. 3312, 3316, 3317, 3329. No tumours grew in any of these birds at the site of injection.

EXPLANATION OF PLATE.

FIG. 1.—GRCH 16. Fowl 3086 original tumour. Spindle cell sarcoma induced with methylcholanthrene. H. & E. × 900.

FIG. 2.—GRCH 17. Fowl 3085 original tumour. Spindle cell sarcoma infiltrating between and invading voluntary muscle fibres which are seen in cross section. Note strap-like interlacing character of tumour cells. H. & E. × 900.

FIG. 3.—Petri dish culture of ascending paper chromatograms. Reading from left to right: GRCH 16 + *B. prodigious*, MH2 + *B. prodigiosus*, sterile control, *B. prodigiosus* control. Incubated 48 hours at 37° C. and allowed to colour on bench for 24 hours.

FIG. 4.—Giant cell and fibroblast reaction tissue around sterile No. 1 Whatman paper pulp injected 198 days previously. Well formed collagen appears black. Van Gieson. × 480.

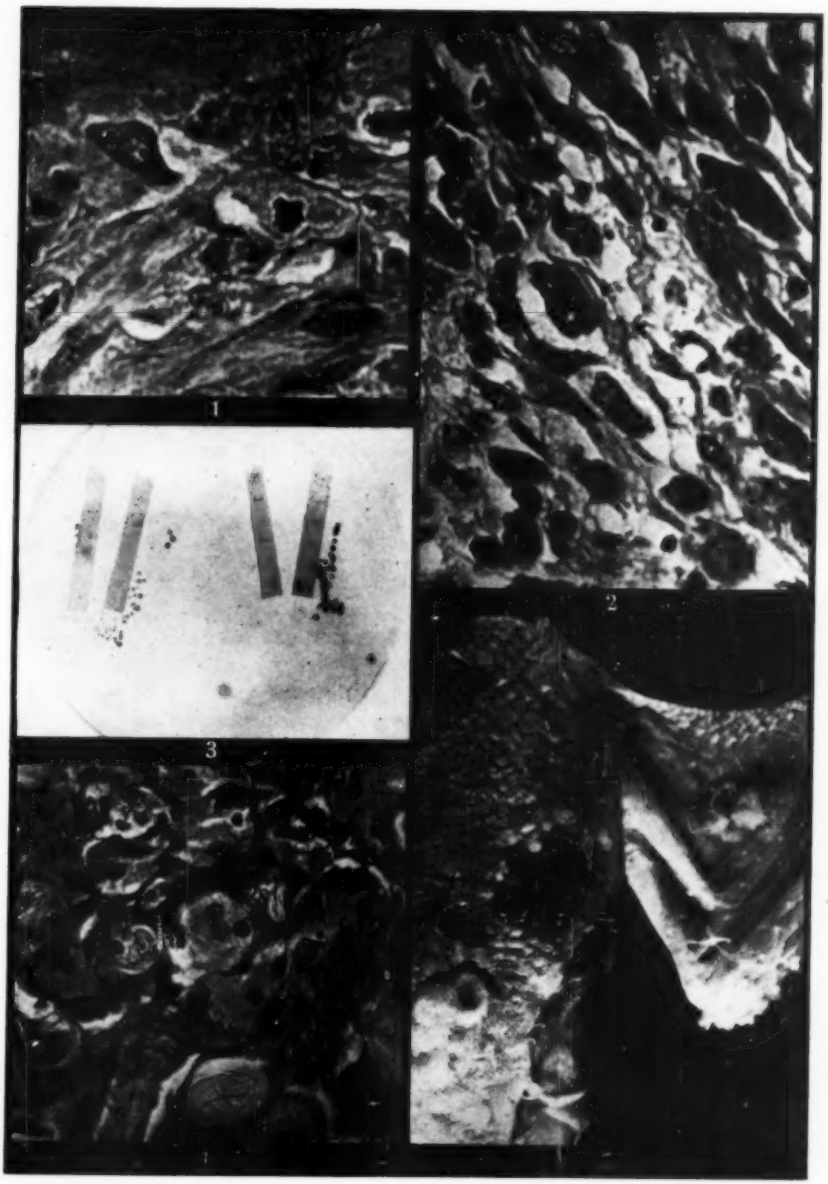
FIG. 5.—Fowl 3309. Rous Sarcoma No. 1 at site of injection in left pectoral muscle region and localisation in left wing web by nitric acid cauterisation 8 days later. The bird was killed 14 days after cauterisation. The skin just above the pectoral tumour was torn accidentally *post mortem*.

FIG. 6.—Fowl 3328. Rous Sarcoma No. 1 injected in right pectoral muscles with reconstituted tumour. Cauterised 6 days later in right wing. Section shows localisation of very early typical Rous sarcoma at site of cautery. H. & E. × 45.

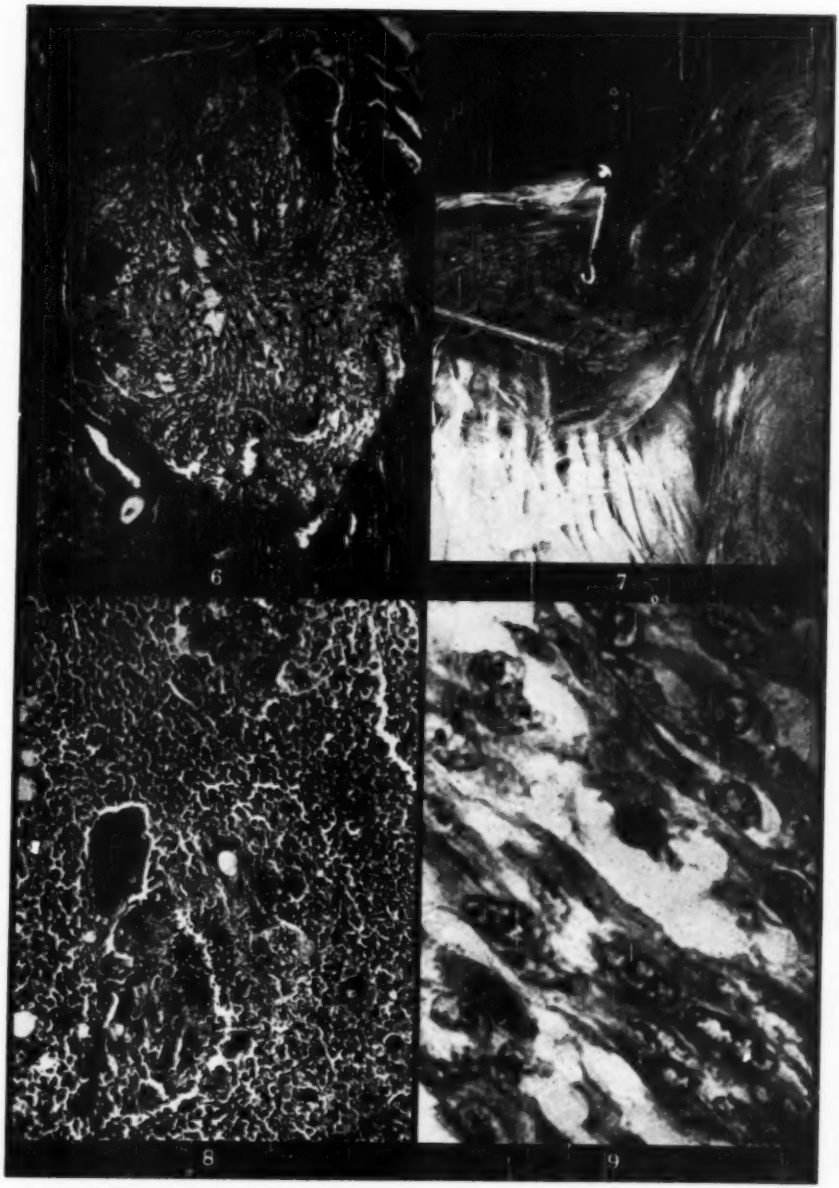
FIG. 7.—Fowl 3896. Injected intraperitoneally with MH2 homogenate. Killed 24 days later with extensive intraperitoneal tumour spread and localised tumour around wing tab. × 1½.

FIG. 8.—Fowl 3896. MH2 endothelioma at site of inflammatory focus around wing tab. A tendency to syncytial growth is characteristic of this tumour. × 170.

FIG. 9.—Fowl 3285. GRCH 17/2 in Rous-immune bird. H. & E. × 1000.



Peacock and Peacock.



Peacock and Peacock.

23.vi.50: No. 3324, showing an early palpable tumour at the site of injection in the pectoral muscles, was cauterised in the right wing with a single thermo-cautery puncture, and in the left wing with a single application of a glass rod moistened with fuming nitric acid.

7.ix.50: No. 3324 was killed. There was a large tumour at the site of injection, but no tumour at either the site of the thermo-cautery or the acid burn, both of which had completely healed within 10 days.

Histologically the tumour was typical GRCH 17 sarcoma. Fresh cell suspension of this tumour was injected into Fowls No. 3312, 3316 and 3317, which had not grown tumours at the site of injection of the Seitz filtrate (see above). Typical GRCH 17 sarcoma grew in No. 3312 and 3316 at the site of injection of cell suspension.

Fresh cell suspension from the tumour in No. 3316 was injected into the left breast of No. 3317, which again failed to grow a tumour.

Thus the GRCH 17 tumour grew in a Rous-immune bird and did not acquire the Rous agent, as far as could be demonstrated by tests in further serial passage.

DISCUSSION.

The undoubted role of viruses in the experimental propagation of a number of malignant tumours in fowls justifies prolonged search for similar viruses in other spontaneous or induced tumours. Over a period of 20 years we have carried out such research for viruses in chemically-induced tumours without obtaining either direct or indirect evidence pointing to such factors as being involved in their aetiology. Nevertheless, it is difficult to exclude the possibility. The peculiar type of localisation associated with proliferative reactions in birds bearing virus-induced tumours has been less easily demonstrable with MH2 endothelioma than with the other virus tumours in our experience. On the other hand, no such localisation has been observed in the case of the GRCH series of tumours.

It seemed that the electron microscope, which can undoubtedly reveal particles of the size of viruses, might afford conclusive evidence of their presence or absence in tumour cells. In our limited experience of this technique, however, there are even less differences to be seen between infective and non-infective tumours and even normal tissues and in homogenates and filtrates prepared from them than can be seen by ordinary visual microscopy or even by direct visual observation. Further work may overcome this initial difficulty of recognising viruses from normal cell constituents; but in the meantime circumstantial evidence points to essential differences between certain induced and spontaneous tumours.

The occasional occurrence of a chemically-induced tumour which on propagation yields a virus does not in our opinion justify the assumption that all tumours contain such viruses. Indeed, the existence of spontaneous filterable tumours would suggest that ultimately one is likely to encounter such agents in birds injected with carcinogens. Duran-Reynals (1952) has recently suggested, however, that viruses not usually associated with tumours may become adapted to cells conditioned by chemical carcinogens, and so play a part in the aetiology of cancer. By painting with methylcholanthrene the skin of birds carrying fowl pox virus he induced histologically malignant epithelial tumours, the cells of which contain characteristic inclusions. Filtrates of such tumours, however,

reproduce fowl pox, not cancer. Our failure to induce any epidermoid cancers in the skin of fowls after repeated painting with chemical carcinogens may be due, as he suggests, to the absence of fowl pox in our flock; but we have had no difficulty in inducing upwards of 60 sarcomata at the sites of injection of chemical carcinogens, and have seen a few carcinomata of the crop following exposure to 2-acetylaminofluorene (Peacock and Peacock, 1949). Though we failed in attempts to transmit the carcinomata and therefore cannot assess their possible viral content, we saw no inclusions of the kind described and illustrated by Duran-Reynals (1952) in his fowl pox methylcholanthrene carcinomata.

The study of a wider range of chemically-induced fowl tumours, preferably including carcinomata of recognisable patterns, seems to offer the best prospects for obtaining more information about the aetiological factors concerned. At the present stage of our knowledge it seems unwise to generalise about viruses in relation to all tumours.

SUMMARY.

1. Three new transplantable, chemically-induced fowl sarcomata, GRCH 16, 17, 18, are described.

2. Mill Hill 2 endothelioma has been successfully transmitted by desiccate kept at 0° C. for 12 years.

3. Comparisons between Rous Sarcoma No. 1, Mill Hill 2 endothelioma and the GRCH 16, 17, 18 sarcomata show that the two former tumours are readily transmissible by filtrates and by other extracts in which cells are probably destroyed or excluded, whereas the GRCH tumours are only transmissible by extracts containing cells.

At the time of writing the authors were unaware that Loewenthal (1931) had used capillary ascent on strips of filter paper of extracts of Rous Sarcoma No. 1 as a means of separating the virus from cells, and achieved the same results as we subsequently obtained.

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THE BIOLOGICAL BEHAVIOUR OF TRANSPLANTS OF THE GRCH/15 FOWL SARCOMA.

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Most cancer investigations involve the use of small rodents, and it is now clear that many of the " generalisations " obtained from research on this material break down when other animals are considered. While the relevance of any result obtained with grafted tumours to the problem of spontanous cancer may be doubted, it seems desirable to report the results obtained by parallel work on non-rodent material in order to offer proof that similar results are, or are not obtained.

The GRCH/15 tumour is a non-filterable fibro-sarcoma originated by Peacock in 1939 (Peacock, 1946), and by his courtesy made available to other workers through the animal Breeding Unit of the British Empire Cancer Campaign. It is readily distinguished from the virus-induced tumours by the absence of the mucoid material that they all produce, but unless the section is specially stained for this, microscopic differentiation is less easy. Transplantation was always made into Brown Leghorns of Dr. Greenwood's flock (in one of which the original was induced), either by implanting small pieces with a fine trocar and cannula, or injection by syringe of the suspended material left after shaking minced tumour or tumour ground with sand and saline. Transplants were usually made into each pectoral muscle, and by the trocar and cannula method " one-sided " negatives occurred about 1 in 20 times, while the second gave less than 1 per 100 " one-sided " failures ; the incorrect negatives due to experimental errors would therefore be 1 in 400 and 1 in 10,000 respectively, which can be ignored. The material for this account is based upon 60 transplant generations involving some 600 birds over a period of 8 years. In most experiments the birds were 6 to 10 weeks old when the tumour was implanted. In the bird the tumour appears as a hard white lump, though after the alteration in the growth rate the texture was softer, and microscopically the cells appeared rather more compact. The tumour only rarely penetrated the skin, but often grew through the sternum to the thorax, producing implantations on the serosa of the heart and liver ; transplants made into one side only frequently eroded through the keel and grew into the muscles of the other side. Birds carrying well-developed tumours usually look rather more anaemic than would be expected from equivalent-sized Rous 1 tumours, and the comb and testes remain juvenile, again unlike Rous 1.

Mutation of the tumour.

During the transplantation of the tumour an abrupt change occurred. This coincided with the removal of the stock to the Experiment Station of the Royal Cancer Hospital of London, and appears to have occurred in the tumour carried

by bird No. 983, from which all material for the 32nd and subsequent generations derived. This bird was inoculated shortly before leaving Edinburgh. It is interesting that the change coincides with a distinct alteration in the conditions of husbandry, for the facilities for keeping animals at the Experiment Station were at first not optimum. No cages were available for birds, and the food comprised various ration-free products, which produced a series of deficiency diseases ranging from protein to multiple vitamin shortages. When the conditions were built up to those nearer first-class management the change in appearance and behaviour of the tumour remained. There is no direct evidence that the change in conditions caused the alteration of the tumour, and it remains possible that it was a coincidence. The most notable change in the tumour was in the rate of growth. The transplants first took about 80 days to fill the breast muscles, and many birds carried their tumours up to 150 days without showing any ill effects. The new rate of growth approximated to that of a Rous 1 tumour, the breast muscles becoming filled in about 30 days and survival over 60 days was rare. Haemorrhages now sometimes occurred in the tumour, but the pink-red fungating mass characteristic of rapid Rous tumours never appeared and no difficulty could be experienced in distinguishing between them macroscopically. Other changes, some of which are dealt with later, were a decrease in the penetration of the thorax, decreased incidence of metastasis to the proventriculus, but, rather interestingly, no decrease in the frequency of takes.

Negative takes.

A genetical basis for the resistence to growth of this tumour has been described by Greenwood and Peacock (1945). In the total of young chicks given this tumour and kept until growths could reasonably be expected to appear, 108 out of 533, i.e., just over 20 per cent proved resistant, while the " rapid " tumour alone gave 94 out of 469 resistant, which is again 20 per cent. This is in marked contrast to the usual finding for rat and mouse tumours and is discussed below.

Peacock (1935) first made the important observation that there was a seasonal variation in the susceptibility of birds to grafts of non-virus sarcomas, and this was confirmed by Murphy and Sturm (1941). Additional data on this variation for the " slow " form of GRCH/15 were given by Greenwood and Peacock (1945). This peculiar effect is still shown by the " rapid " form (Table I), even though this data is biased by deliberate attempts to avoid large-scale experiments during the period when successful takes are minimal.

TABLE I.—*Seasonal Variation in Frequency of Negatives of " Fast " Tumour Strain.*

Year.	Jan.	Feb.	Mar.	Apr.	May.	June.	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.
1947 . . .									2/3	0/4	1/6	4/7
1948 . . .	3/7	—	0/6	5/13	1/11	2/17	3/14	2/12	5/12	6/19	3/21	4/23
1949 . . .	3/44	3/43	5/19	0/16	3/9	1/9	4/19	1/10	5/9	5/15	6/10	1/7
1950 . . .	4/11	1/6	1/12	2/8	3/17	0/14	2/6	3/10				
Total . .	10/62	4/49	6/37	7/37	7/37	3/40	9/39	6/32	12/24	11/38	10/37	9/37
Per cent. .	16	8	16	19	19	7	23	19	50	29	27	24

Effects of tumour on host.

The figures for these effects are only approximate, for two reasons; firstly a proportion of the tumours, always the faster-growing ones, were sent to other

workers, and secondly, those cases in which the tumour penetrated the thorax with resulting generalised sarcomatosis are ignored. Both therefore include the most rapid and malignant tumours, so that the estimates given subsequently are *minimal* figures.

1. *Metastasis.*—A striking characteristic of the GRCH/15 sarcoma is the frequency of location of metastases in the proventriculus. By contrast, in only one bird out of many thousands has this organ been the site of a Rous 1 metastasis. Of 352 birds killed and examined with large growths in the breast muscle, 51 had metastic growths in one or more sites. The actual frequency of metastasis probably differed somewhat in the " slow " and " rapid " tumours, and is shown in Table II. In only 6 of these birds were the lungs and proventriculus found

TABLE II.—*Distribution of Metastases.*

Type	No.	Proventriculus.	Lung.	Liver.	Heart.	Mesentery.	Ovary.	Others.
Slow	18	12	9	5	3	4	0	0
Fast	33	14	23	7	1	2	0	Kidney, gizzard
Adult (slow)	18	4	15	2	0	1	4	0

to be free from metastases while, as might be expected, heart secondaries were always associated with widespread dissemination. The earliest record of a metastasis is after 29 days for the " fast " tumour and 36 for the " slow ", and they usually occurred at an average of about 40 and 50 days respectively. The " fast " tumours, as far as the records go, seem less prone to develop secondaries, but this is probably in part due to the fact that many were killed at a slightly earlier stage of development than the " slow " tumours.

2. *Effect on liver.*—About half the birds bearing large GRCH/15 tumours show a grossly enlarged liver, congested and thickened, with rounded edges. This was diagnosed by Dr. J. G. Campbell of the Poultry Research Centre as passive chronic venous congestion and early diffuse generalised fibrosis. About half of these birds show enlargement of the spleen as well, and about one-third of those with enlarged livers develop ascites with the " fast " tumour, and rather more with the " slow " tumour. This reaction appears to be related to the amount of tumour present rather than to the duration of growth, for liver reactions were reduced or absent in hosts with slow tumours even when kept for longer periods, and were less frequent in those birds with tumour in only one breast. Over half a litre of clear ascitic fluid was removerd from a bird on one occasion, and when the condition was well advanced it was necessary to tap more frequently than once per week. The number of birds showing pathologic livers was about equal in the " fast " and " slow " tumour groups, but while the former usually died of lung metastases, the latter often succumbed to conditions associated with the enlarged liver, such as compression of the great blood-vessels, or of the heart.

Growth in adult females.

Twenty-four laying females were inoculated with the " slow " tumour in each breast. They were all from a cross between two particular lines (BP × INT); the resistance of these lines was 57 per cent and 40 per cent respectively in the report of Peacock and Greenwood (1945). Eighteen of these developed metas-

tases, fifteen of which were in the lungs and only four in the proventriculus. A special feature was that 4 developed metastases in the ovary, an organ that was never affected when immature birds were used. Most birds ceased laying 2 to 3 weeks after inoculation, so that if metastasis takes place in the functioning ovary, it must occur rather early (cf. above 36 days minimum and 50 days average for visible secondaries in immature birds with the " slow " tumour).

Intravenous injection with cells.

Mainly with the intention of determining whether the localisation of metastases in the proventriculus was due to preferential filtration of the cells by this organ, or to the fact that it was a more congenial place to grow, fine suspensions of cells from a " slow " tumour were injected intravenously. In two experiments only one-third of the birds developed tumours, and these also formed tumours at the site of the injection. The results could not therefore br related to the number of viable tumour cells inoculated (as determined by titration of the suspension in other birds). The proventriculus was, however, always involved, and the tumours there were much larger than those in other sites (lung, liver, etc.). Indeed, the proventriculus was sometimes almost completely replaced by several tumours coalescing so that they were difficult to separate and count, while the others were difficult to count because they were barely detectable as yet. Clearly the trapped cells grow much faster in the proventriculus than elsewhere.

Inoculation of the tumour tissue with proventriculus tissue from the same donor did not give enhanced growth, and no indication of survival of the normal tissue was seen.

Attempted transmission by cell-free preparations.

That the tumour is not transferred other than by cells was demonstrated in a long series of careful experiments by Peacock (1946). Here it may be simply mentioned that this was fully confirmed in various experiments, using glycerinated tissue (3 experiments), cells lysed by hypotonic solutions (2 experiments), freeze-dried tumour tissue (2 experiments), and macromolocular preparations derived by techniques which yield active Rous virus (4 experiments).

Neutralisation of Rous virus.

Six experiments were made to determine whether antibodies to Rous 1 virus were developed by birds growing GRCH/15 tumour. In all, sera from 3 birds resistant to inoculation and re-inoculation of GRCH/15 cells were tested, using normal fowl serum as the control. In addition, 5 tumours of more than 2 months' growth were glycerinated, and the result extracted with water, clarified, and tested using a similar extract of mouse liver as control (this method easily detects serum antibodies contained in the blood of Rous 1 tumours (Carr, 1944)). In only one test was any neutralisation detected. As natural antibodies were expected to be an occasional complication, the anomalous result was attributed to this, and the other seven negatives taken as proof that GRCH/15 does not itself produce antibodies to Rous virus.

DISCUSSION.

It is at once apparent that many of the results reported for the GRCH/15 sarcoma, though agreeing with the findings of other workers upon fowl tumours,

are in contradiction to the results obtained with the lower rodents. Since for most purposes the aim of cancer research is directed towards the understanding and control of the human disease, it is important to note at which points the results for rodents cannot safely be used for generalisations.

The most important of these differences is the finding, first appreciated by Peacock (1935) and confirmed by Murphy and Sturm (1941), that the fate of a tumour graft in the fowl is determined only partly by the genetically-controlled tissue antigen compatibility, but mainly by the season. The same occurs with induced fowl teratomas (Michaelowsky, 1928 ; Falin and Gromzewa, 1939 ; Bagg, 1936). The present finding that a fast mutant of the GRCH/15 is still controlled to a great extent by this influence serves to emphasise this aspect. This cannot be due to inability of the fowl to produce antibodies, for it is at least as good as the rabbit in this respect, and much superior to the rat or mouse. Nor is it due to a failure of the bird to produce iso-antigens. The conditions for tissue transplantation, however, seem to differ markedly from those of the lower rodents. For example, organ transplantation between fowl varieties is a normal experimental technique, used for example by Greenwood (1928) to investigate the relation of the testis to henny feathering of males of some breeds ; yet transplantation of an adrenal is regarded as a novel event in the mammalian field (Darcy, 1952). Similarly, the elaborate integumental decorations of birds early attracted the attention of zoologists, and the results of skin transplant between different *genera* were being embodied into the general assembly of zoological theory before tissue iso-antigens were recognised.

It is possible to suspect that a similar mechanism is indeed operative in the mammalian field, but the methods used are not refined enough to detect it. The domesticated rat and mouse have an oestrous cycle of only a few days in length, as compared with the sharply-defined annual cycle in the hen, and lesser cyclic variation of the cock. Even the pregnancy of 3 weeks is rather short for observations in the rat and mouse, but it is significant that Foulds (1949) found some evidence of a variation in tumour growth and structure in the mouse, and there are many reports of pregnancy influencing the course of tumours in the human. The appearance of many cancers at an age when the sexual functions are failing may be another aspect of this.

This would seem to be a point of considerable importance, for it indicates that control of a malignant tumour would be possible within the limits of normal physiological variations ; similarly studies on the cancers induced by chemicals, and more especially by X-rays, indicate that the production of the malignant change and the appearance of a progressive tumour are distinct phenonema, a separation that is even more obvious with the mouse milk factor, where infection takes place soon after birth but the tumour appears months later, and only if the endocrine history of the host has been suitable. The fowl obviously offers very favourable material for the study of the factors whereby the host susceptibility alters.

The difference between the frequency and distribution of metastases in immature and mature fowl is again in contrast to the rodent findings, but mostly because this aspect has not been investigated in the latter. There are some suggestions that this factor operates in human cancer, but since this necessarily concerns cancers originating from cells of different age as well, it cannot be regarded as referring to the host alone. Whether the decrease in the frequency of second-

aries in the proventriculus in older birds carrying the "slow" tumour refers to a difference in the biochemical suitability of the organ in the older hosts is unknown. It is to be expected that the differences of tissue permeability with age (Duran-Reynals, 1942) would also have some influence upon the metastatic growths.

The absence of any neutralising action in the sera of fowls immunised against the GRCH/15 sarcoma is in contrast to the findings of Gottschalk (1943) for Sarcoma 16. But it is well known for commercial birds suffering from the related "leukosis complex" viruses to have death-rates of up to 40 per cent and carrier rates of nearly 100 per cent, so that such a finding is only of value if it can be stated that such carriers are not a complicating factor. The disease and carriers are known to be very rare in the Edinburgh flock (the solitary neutralisation found against the Rous 1 virus may have been such). While Gottschalk (1943) gave no data for this, he frequently refers to cases of leukosis arising in his experimental material, and his normal fowl sera frequently contained neutralising antibodies. It would therefore seem wise to suspend judgment upon his conclusion that Sarcoma 16 contained an antigen related to tumour-reducing viruses not present in normal cells until any suspicion of this complication is removed.

SUMMARY.

This account of the properties of the chemically-induced GRCH/15 fowl sarcoma is based upon 60 transplant generations over 8 years. The deciding factor for growth depends as much upon the season as upon the tissue compatibility. A fast-growing mutant strain retained this property, and the mutation was not associated with an increased frequency of takes, though the distribution of secondaries was altered. The distribution of secondaries was also affected by the age of the host. The proventriculus was a favourite site for metastases, and the tumour grew more rapidly in this site than elsewhere.

No antibodies against Rous 1 virus were produced in immune fowls.

The differences between the results of this tumour and those obtained for tumour transplants in small rodents are emphasised and discussed.

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THE OPTICAL FORM OF GLUTAMIC ACID IN TUMOURS.

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In 1939 Kögl and Erxleben (1939*a*) published the first of a series of papers on the optical configuration of amino-acids in proteins from tumours. It was found that the optical rotations of some amino-acids, which had been isolated by the classical methods and purified by recrystallisation, did not correspond with the values in the literature. It was concluded that the preparations contained the "unnatural" D-isomer in addition to the "natural" or L-isomer. The calculated proportion of D-isomer was largest in serine and glutamic acid. The authors considered that serine had probably been partly racemised during hydrolysis of the proteins or during subsequent purification. Glutamic acid on the other hand was considered resistant to racemisation in acid solution. Glutamic acid from normal tissues and benign tumours had the specific rotation $[\alpha]_D^{20^\circ}$ + 31·6°. Attention was directed to this apparent difference in the optical form of glutamic acid from malignant and non-malignant tissues, which promised to be a lead to the long-sought chemical difference between normal and neoplastic tissue, and one that could be used for diagnostic purposes. A theory of malignity was proposed (Kögl, 1939) depending on the inability of most peptidases to hydrolyse peptides of D-amino-acids.

The experiments of Kögl and collaborators were repeated in many other laboratories with variable results, which on the whole showed smaller amounts of D-isomer than had been found by Kögl. An especially thorough investigation was made by Klingmuller (1943). The considerable controversy which developed between different schools was suspended by the recent war, but Dodds and Dickens (1940) had already concluded from a review of the results then available that the appearance of the D-isomer of glutamic acid was not characteristic of tumour tissues. Since the war further work by Kögl, Barendregt and Klein (1948) and Kögl (1949, 1950) has renewed discussion of this problem. Indirect evidence was produced in support of the original hypothesis. It was decided to limit the present study to the problem of whether or not the D-isomer is found in protein hydrolysates, and if so whether it exists as such in the proteins or is an artifact. A similar study using different methods (Boulanger and Osteux, 1950) has appeared since the experimental work was completed.

Preliminary experiments (Wiltshire, 1953) showed that glutamic acid is racemised to the extent of about 5 per cent in the preparation of protein hydrolysates. Normal plant and animal protein hydrolysates contained up to 6 per cent of the D-isomer. Kögl and Erxleben (1939*b*) claimed that the proportion

of D-isomer in malignant tumour proteins was much higher than this, ranging from 15·6 to 44·5 per cent. Of the D-isomer 5 per cent or less was regarded by them as insignificant, and the same lower limit has been accepted in the present work.

MATERIALS.

Calf lung protein.—This was the same dried and powdered preparation analysed by Chibnall, Rees, Williams and Boyland (1940), and fully described in that paper.

Rous sarcoma.—This was dried and powdered tissue prepared in the same way. It contained 13·77 per cent N uncorrected for moisture or ash.

Jensen sarcoma.—Of fresh tissue 63·5 g. was homogenised with 64 ml. of 0·11 N sodium chloride solution, 380 ml. 0·11 N sodium chloride was added and the suspension stored at 0° for 36 hours. The suspension was filtered. Soluble protein in the filtrate was precipitated by addition of 1200 ml. of 95 per cent ethanol, centrifuged, washed twice with ethanol and twice with ether, dried at 110° and weighed. The whole preparation (2·524 g.), containing 14·08 per cent N when dry, was hydrolysed.

Carcinoma of bronchus.—A histological description and chemical analysis of this material was given by Chibnall, Rees, Williams and Boyland (1940).

Carcinoma of colon.—This preparation consisted of metastases in the liver of a man with carcinoma of the colon.

Pathologist's report (from Dr. A. C. Thackray, Bland-Sutton Institute of Pathology, Middlesex Hospital, London): "There was a tumour at about the middle of the transverse colon. The liver was very greatly enlarged, weighing 208 ounces, and was almost entirely replaced by yellowish-white nodules of secondary growth up to 3 inches in diameter; those reaching the surface of the organ were umbilicated. Microscopical examination showed the tumour to be a columnar cell adenocarcinoma with areas of mucoid degeneration and necrosis."

The tumours were dissected from the surrounding tissue and weighed. One volume of water and a little toluene were added and the whole homogenised in a Waring blender.

The suspension was stored at 0° for 48 hours. It was then centrifuged, giving a supernatant of soluble proteins *A* and a residue of insoluble material *B*. *B* was extracted twice with acetone, dried, ground and weighed. The powder was analysed as insoluble protein (Table I). To *A* were added 4 volumes of ethanol

TABLE I.—*Isomers of Glutamic Acid in Normal and Neoplastic Tissue Proteins.*

Tissue.	Glutamic acid N as per cent total N. 1. Both isomers (mean).	2. L-isomer (mean S. e.).	3. D-isomer by difference.	4. D-isomer as per cent total glutamic acid.
Normal calf lung	5·69	5·49 ± 0·08	0·20	3·5
Rous sarcoma	7·03	7·07 ± 0·08	*nil*	*nil*
Jensen sarcoma	9·22	8·73 ± 0·08	0·49	5·3
Carcinoma of bronchus	6·23	6·29*	*nil*	*nil*
Carcinoma of colon:				
Insoluble protein *B*	7·10	6·62 ± 0·05	0·48	6·8
Soluble protein *C*	7·71	7·89 ± 0·04	*nil*	*nil*
Amino-acids *D*	4·65	4·58 ± 0·11	0·07	1·5

* Insufficient estimates for statistical analysis.

to precipitate the water-soluble protein *C*. The precipitate *C* was extracted with acetone, ethanol and ether and dried. The filtrate and washings from *C* were combined with the washings from *B* and concentrated at 40° under reduced pressure to give a residue *D*. This contained lipids, salts, free amino-acids and small peptides. *D* was extracted with ether in a Soxhlet apparatus, and the ether-insoluble part analysed.

METHODS.

Estimation of glutamic acid isomers.—The procedures for estimation of L-glutamic acid and D-glutamic acid have been described elsewhere (Wiltshire, 1953).

RESULTS.

Neither the normal protein used as a control nor any of the 4 tumours analysed contained more D-glutamic acid than could have been formed from the L-isomer during preparation of the hydrolysates (Table I).

DISCUSSION.

The results of analyses by the modern method used in this study correspond closely with those obtained by Chibnall, Rees, Williams and Boyland (1940) by isolation on the same materials. Thus Chibnall *et al.* found for calf lung protein total glutamic-N equivalent to 5·59 per cent and the L-isomer to 5·54 per cent of the protein-N compared with the present 5·69 per cent and 5·49 per cent respectively. For the carcinoma of the bronchus they found 6·40 per cent and 6·29 per cent compared with the present 6·23 per cent and 6·29 per cent. Both methods therefore would appear to account for all the glutamic acid of the proteins. This is of some importance, since in much of the earlier work involving purification of the amino-acid for polarimetry a very small proportion of the total glutamic acid was finally isolated and analysed. The same criticism may be made of the alumina-chromatographic methods of Wieland (1942) and Boulanger and Osteux (1950), where only 70 to 80 per cent of the original glutamic was recovered. The isolation of a small and unrepresentative sample may account for the discrepancy between the results of experiments by Graff, Rittenberg and Foster (1940) and Wieland and Paul (1944), who, using N^{15}-labelled glutamic acid, could find only 2 to 5 per cent D-isomer in the glutamic acid from tumours, and by Kögl, Erxleben and Van Veersen (1943), who with Deuterium-labelled glutamic found over 20 per cent. The latter experiments have been criticised on other grounds by Rittenberg and Shemin (1946).

Soon after the discovery of D-amino-acids in tumours several workers tested the activity of D-amino-acid oxidase from kidney on hydrolysates of tumour and other tissue proteins. Among others Kögl, Herken and Erxleben (1940); Lipmann, Behrens, Kabat and Burk (1940); Lipmann, Hotchkiss and Dubos (1941); Arnow and Opsahl (1940*a*, 1940*b*) and Boulanger (1944) could find no D-amino-acid in whole hydrolysates by this means. Yet Kögl, Herken and Erxleben (1940) found that their "racemised glutamic" preparations from tumours were oxidised by the kidney enzyme. Since Klein and Handler (1941) and more recently Bender and Krebs (1950) have shown that kidney D-amino-acid oxidase does not oxidise D-glutamic acid, it may be inferred that the "racemised glutamic" preparations contained some other amino-acid.

In a recent paper Kögl (1949) attributed the failure of other workers to confirm the discovery of D-glutamic acid to insufficient hydrolysis. If the tumour proteins were hydrolysed by the method generally used for pure proteins, i.e., by refluxing for 24 hours with 6N hydrochloric acid, " auch wir nur einen Totalwert von durchschnittlich 10·5% Glutaminsäure mit einem übertrachtlichen Gehalt an D-form . . . (erhielten)." It was stated that the D-isomer could be released from peptide bonds only by hydrolysing with 10N acid on an oilbath at 135 to 160°. All the D-isomer was released in the first 7 hours; further hydrolysis up to 20 hours raised the yield of the L- but not of the D-isomer. These special hydrolysis conditions were adopted by Boulanger and Osteux (1950), but apart from a little more racemisation at the higher temperature (found also with normal proteins) there was no more D-isomer found than when the protein was hydrolysed in the normal way.

There is evidence that D-amino-acid oxidase activity varies under some physiological and pathological conditions. It is said to be lowered in rats bearing certain tumours and in normal pregnant females (Tien Ho Lan, 1943; Westphal, 1942, 1943, 1944). The first effect of such decreased activity might be the accumulation of D-amino-acids in the body fluids of such animals. For this reason D-glutamic acid was sought in the free amino-acid fraction of the material from carcinoma of the colon, but none was found.

SUMMARY.

One normal tissue and four tumours were analysed for the isomers of glutamic acid by the methods previously applied to purified proteins. The percentage of D-glutamic acid found was in all cases very small, and not more than would be formed by inversion of the L-isomer during hydrolysis.

I wish to thank Dr. B. Holmes for the Jensen sarcomata, the Bland-Sutton Institute of Pathology, Middlesex Hospital, London, for the liver metastases from a case of carcinoma of the colon, and Professor F. Dickens for help in this work. I am most grateful to Professor A. C. Chibnall for suggesting this problem, and for his help and advice.

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THE IMMEDIATE EFFECT OF SOME PHOSPHORYLATED NAPHTHOHYDROQUINONES ON DIVIDING CELLS.

A Study on Living Cultures of Chick Osteoblasts.

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In this paper are described the short-term effects of 2-methyl-1:4-naphthohydroquinone diphosphate and two related compounds on living cells in division within cultures of osteoblasts from chick embryos. These effects have been studied by means of phase-contrast cine-micrography in the manner which has been described by one of us (Hughes, 1949*a*, 1949*b*), and used in similar studies of the influence of a number of other inhibiting substances on dividing cells (Hughes and Fell, 1949; Hughes, 1950, 1952*b*).

"Short-term effects" are here understood as those which are seen during the course of a single cycle of cell division. The substance, usually dissolved in Tyrode saline, is added to a culture after a dividing cell in mitosis therein has been selected for study, and the reaction of that cell to the agent is recorded for a period usually less than one hour. Where, however, the aim is to subject a dividing cell to the influence of a substance as early as possible in the course of mitosis the procedure has been reversed, and the culture was treated before placing under the warm-stage microscope. Usually several minutes elapse before s suitable cell in early prophase is then found.

In order to examine whether these substances affect the entry of resting cells into mitosis under the conditions of these experiments, batches of cultures were treated with appropriate dilutions of these compounds, and were fixed after a period of 60 minutes. The cells in prophase then to be found in the outgrowth of the stained cultures were subsequently counted.

The effects on mitosis of three phosphorylated naphthohydroquinones have been studied in this work. These are as follows:

1. Tetra-sodium, 2-methyl-1 : 4-naphthohydroquinone diphosphate (Synkavit, Compound I).
2. Tetra-sodium, 2:3 dimethyl-1 : 4-naphthohydroquinone diphosphate (the dimethyl derivative, Compound XXVIII).
3. Tetra-sodium, 1:4-naphthohydroquinone diphosphate (the methyl-free derivative, Compound II).

These compounds will here be referred to by the same numbers as are given in Mitchell and Simon-Reuss (1952*a*, 1952*b*).

The selection of these three compounds was based on previous research on radiosensitising agents in connection with the chemotherapy of cancer. Mitchell

in 1946 first studied the therapeutic effect of Synkavit in combination with irradiation in cancer patients, and later gave evidence with Simon-Reuss (Mitchell and Simon-Reuss, 1947) that this compound can inhibit mitosis in chick fibroblasts and also in some human carcinomata. These authors also showed that it can enhance the effect of irradiation.

Further studies on the relationship between chemical structure and biological action led to the investigation both of the methyl-free derivative (Friedmann, Marrian and Simon-Reuss, 1948) and the di-methyl compound (Mitchell and Simon-Reuss, 1952*a*, 1952*b*).

In tissue cultures the methyl-free substance appeared to be the strongest inhibitor of the three, though it was without effect either in experiments on the whole animal, or in clinical trials. The di-methyl compound was found to be slightly less active than Synkavit as a mitotic inhibitor *in vitro*. It again potentiates the effects of irradiation. Further work on this substance is in progress, including clinical trials.

Normal Mitosis in the Chick Osteoblast.

Osteoblasts were chosen for this work on the grounds that clear phase contrast pictures of them can be obtained during mitosis, and that they appear to be much less sensitive to light than are other fibroblast types of chick cells.

In previous papers there has been described the normal course of cell division which can be observed under the phase-contrast microscope in living osteoblast cultures (Hughes and Fell, 1949; Hughes, 1949*b*). The nucleoli and nuclear membrane disappear remarkably rapidly at the end of prophase, but while the spindle is being formed several minutes pass before the chromosomes assume their equatorial position as a metaphase plate. It is convenient to speak of all this period after the loss of nuclear membrane and nucleoli as metaphase, and to separate an early from a late division of this phase of mitosis, respectively before and after establishment of the metaphase plate.

The General Cytological Effects of Compounds, I, II, and XXVIII.

In the present work over fifty film records of the behaviour of dividing cells under the influence of these substances have been made. The analyses of these has yielded the data which are presented in Table I, II and III. In the description of the observations which follows the bracketed numbers refer to items from these tables.

The most common inhibitory action of the methylated compounds are those which cause arrest of mitosis during metaphase (Fig. 1–5; Fig. 12–19). Either the mitotic spindle is not formed, or if already present it is disorientated; the chromosomes clump together into one or more amorphous masses. With Synkavit (I), in addition, the whole cell becomes oedematous, though this effect is also seen both in cells which continue division and among those in the resting state (Mitchell and Simon-Reuss, 1952*a*, 1952*b*). In the latter, however, the nucleus is unaffected, while in cells inhibited during mitosis the degree of oedema in the cell as a whole influences the behaviour of the clumped chromosomes, which in much swollen cells continuously change their position (Fig. 1–5). Synkavit causes more cell oedema under the conditions of these experiments than does the

di-methyl compound, but in instances where the mitotic spindle is disorientated by the latter substance the clumped chromosomes become widely scattered throughout the cell.

EXPLANATION OF PLATES.

One cell in each group of Figures was photographed during life at the stated intervals by phase contrast during the course of an experiment and, except in the last group, finally after fixation and staining. Magnification × 2000 in all.

FIG. 1–5 (Table I, No. 10) : The effect of 7×10^{-6} M Synkavit (I) on a cell in metaphase.

FIG. 1.—One minute after addition.
FIG. 2.—9½ minutes later than Fig. 1.
FIG. 3.—13½ minutes later than Fig. 1. The chromosomes have clumped together.
FIG. 4.—21 minutes later than Fig. 1.
FIG. 5.—After fixation and staining. Culture fixed 25 minutes later than Fig. 1.

FIG. 6–9 (Table II, No. 44).—The effect of the di-methyl compound (XXVIII) at 8×10^{-6} M on a cell in metaphase.

FIG. 6.—One minute after addition.
FIG. 7.—9 minutes later than Fig. 6. Cell now in anaphase.
FIG. 8.—34 minutes later than Fig. 6. Cell still in anaphase. Both cleavage and nuclear reconstruction have been inhibited.
FIG. 9.—After fixation and staining. Culture fixed 36 minutes later than Fig. 6.

FIG. 10, 11 (Table I, No. 53).—The effect of 5×10^{-7} M Synkavit (I) on a cell in prophase.

FIG. 10.—10 minutes after addition. Nucleoli and nuclear membrane are present.
FIG. 11.—After fixation and staining 20 minutes later. All still in same condition.

FIG. 12–15 (Table II, No. 46).—The effect of the dimethyl compound (XXVIII) at 2×10^{-6} M on a cell in prophase.

FIG. 12.—11 minutes after addition. Nucleoli and nuclear membrane still present.
FIG. 13.—5 minutes later than Fig. 12. End of prophase.
FIG. 14.—19 minutes later than Fig. 12. The chromosomes have clumped together.
FIG. 15.—Cell after fixing and staining. Fixation 21 minutes after Fig. 12.

FIG. 16–19 (Table I, No. 63). The effect of 4×10^{-6} M Synkavit (I) on a cell in prophase.

FIG. 16.—One minute after addition. Nucleoli still present.
FIG. 17.—9 minutes later than Fig. 16. Late prophase.
FIG. 18.—32 minutes later than Fig. 16. The chromosomes have clumped together.
FIG. 19.—Cell after fixing and staining. Fixation 35 minutes after Fig. 16.

FIG. 20.—Cell from the outgrowth of a culture treated with 5×10^{-7} M Synkavit (I) for 40 minutes. Cell has undergone mitosis with the suppression of cleavage. The daughter nuclei are in late telophase.

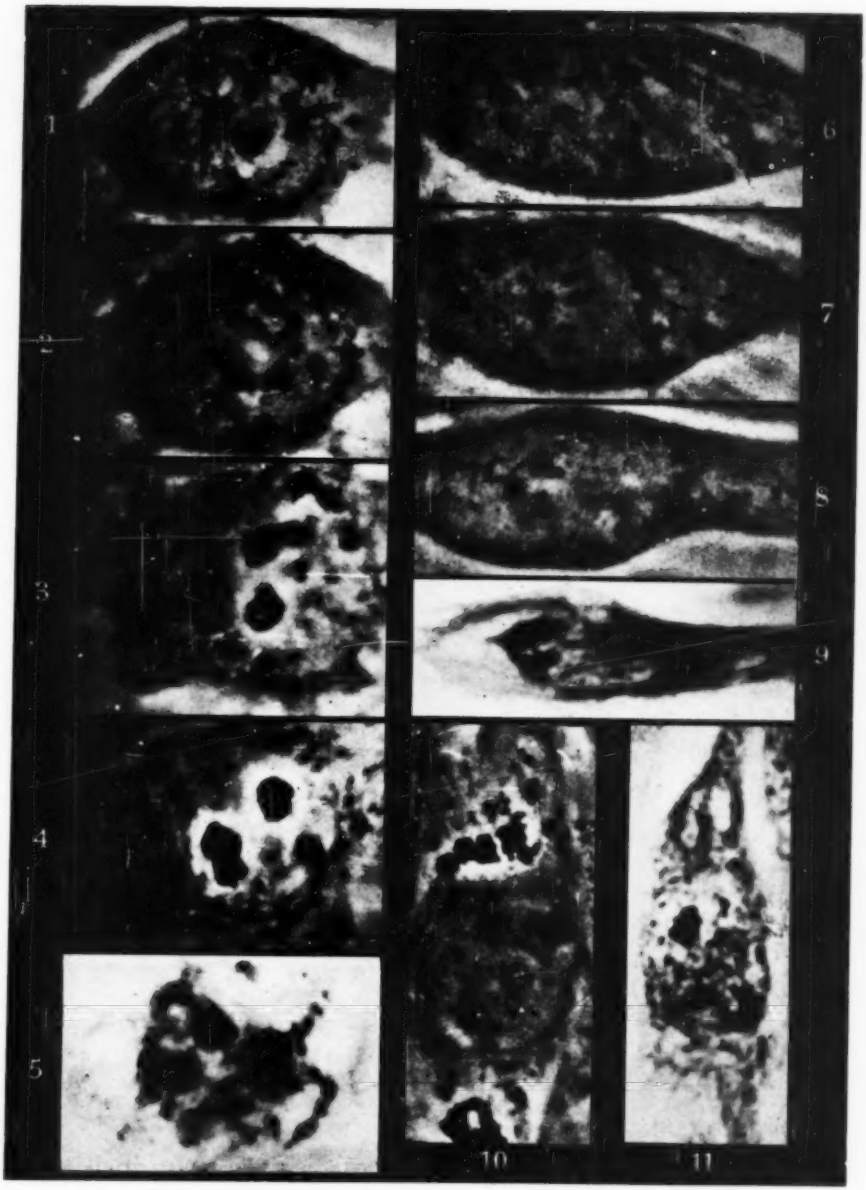
FIG. 21.—Cell in the outgrowth of the same culture as in Fig. 20. The chromosomes have clumped into several scattered groups at some period after prophase.

FIG. 22–26 (Table III, No. 41).—The effect of the methyl-free compound (II) at 5×10^{-6} M on a cell in early metaphase.

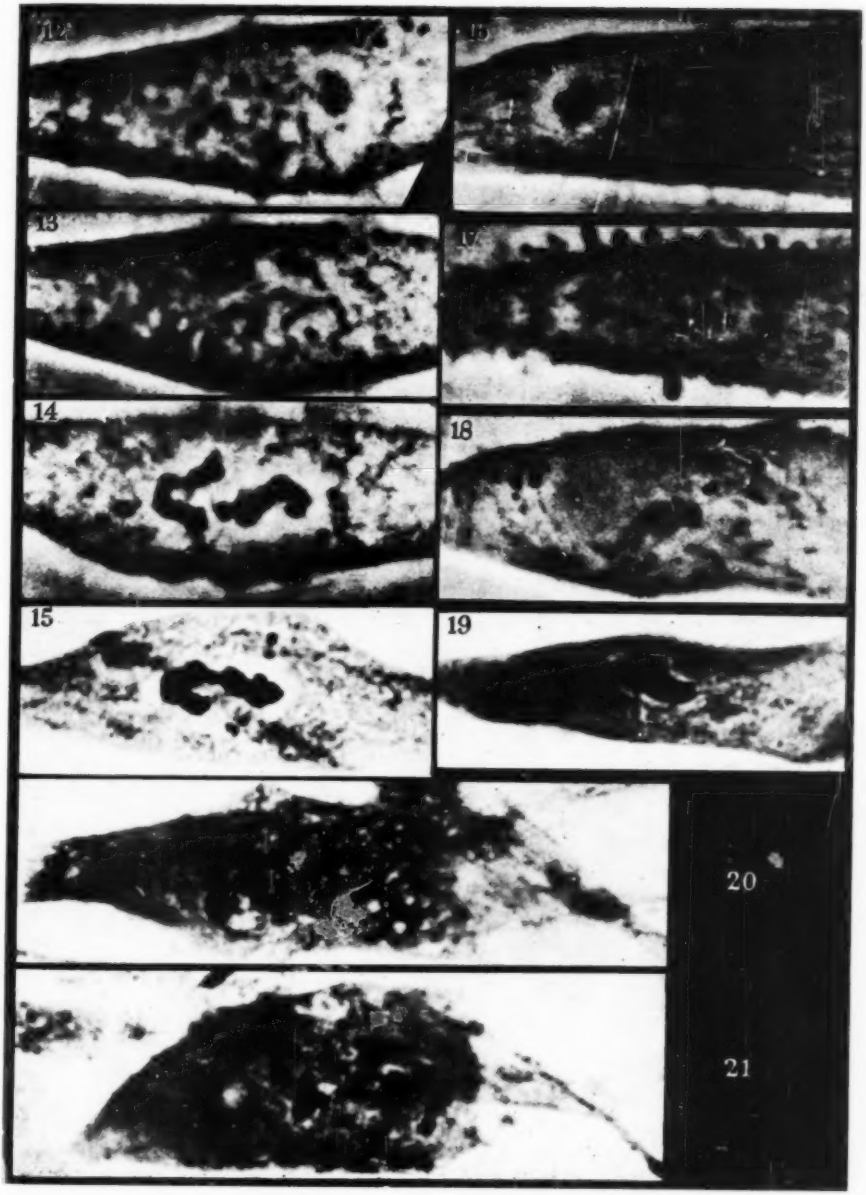
FIG. 22.—3 minutes after addition.
FIG. 23.—23 minutes after Fig. 22. Early anaphase.
FIG. 24.—48 minutes after Fig. 22. Cell bubbling vigorously without cleavage.
FIG. 25.—57 minutes after Fig. 22.
FIG. 26.—After fixing and staining. Fixation 63 minutes later than Fig. 22. Daughter nuclei have reconstruction but the cell has not divided.

FIG. 27–31 (Table III, No. 31).—The effect of the methyl-free compound (II) at 1×10^{-6} M on a cell in metaphase.

FIG. 27.—One minute after addition.
FIG. 28.—10 minutes after Fig. 27. Early anaphase.
FIG. 29.—12 minutes after Fig. 27. Normal cleavage.
FIG. 30.—24 minutes after Fig. 27. Daughter cells begin to re-unite.
FIG. 31.—42 minutes after Fig. 27. Complete re-fusion.



Hughes and Simon-Reuss.



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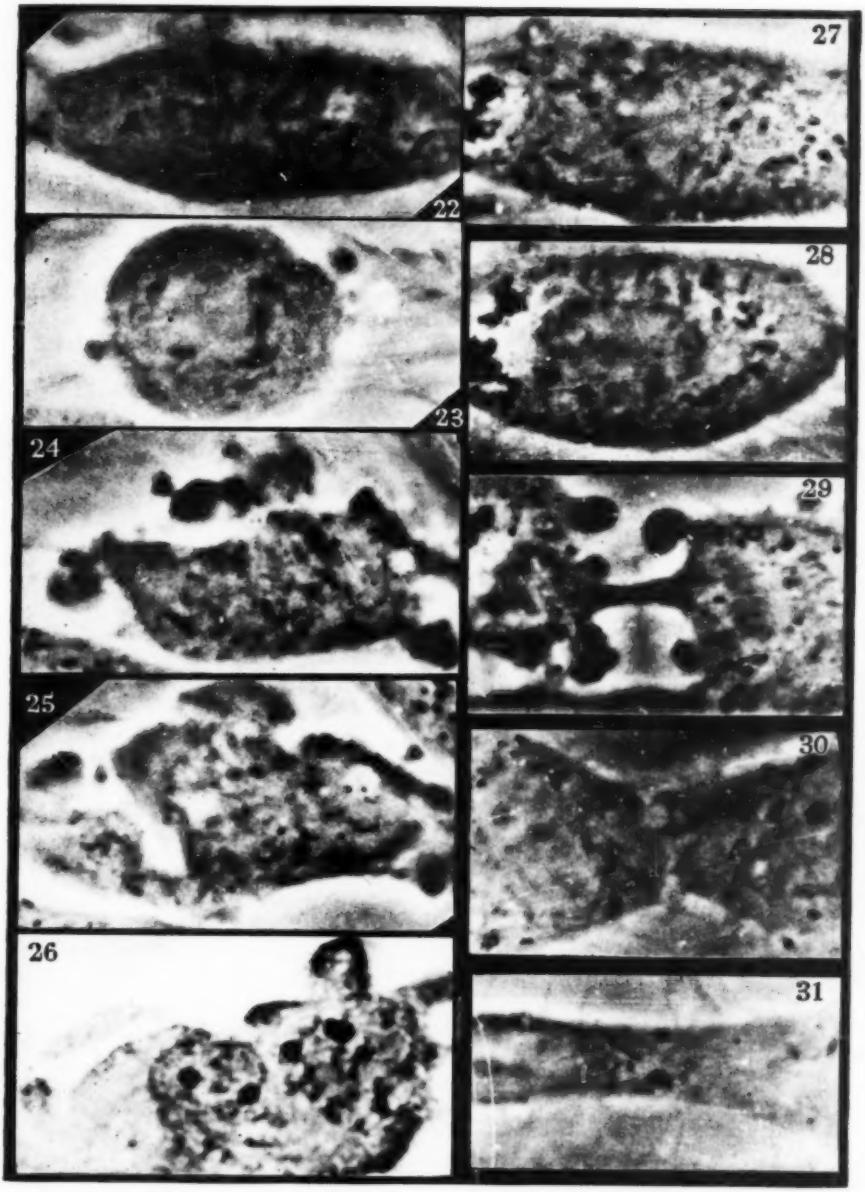

Hughes and Simon-Reuss.

TABLE I.—*Synkavit (I).*

Ref. No. of cell.	Concentration.	Stage applied.	Effect.
56	5×10^{-7} M	Prophase. Nucleoli still present 5 min. later	Normal mitosis.
57	,,	Ditto, ditto, 5 min. later	,, ,,
55	,,	,, ,, 8 ,,	,, ,, but cell oedema.
53	,,	,, ,, 10 ,,	Stays in early prophase for further 20 mins.
59	1×10^{-6} M	,, ,, 7 ,,	Normal mitosis.
60	,,	,, ,, 9 ,,	Complete mitosis but delayed in metaphase.
49	,,	,, ,, 10 ,,	Stays in early prophase for further 20 min.
52	,,	,, ,, 11 ,,	Chromosomes clump, cell oedema.
50	,,	Late prophase	Ditto.
61	2×10^{-6} M	Prophase. Nucleoli still present 5 min. later	Normal mitosis but cell oedema
62	,,	Ditto, ditto, 10 min. later	Chromosomes clump, cell oedema.
17	$3{\cdot}5 \times 10^{-6}$ M	Metaphase	Completes mitosis.
18	,,	Metaphase plate	,, ,,
19	,,	Metaphase	,, ,,
20	,,	Late prophase	Chromosomes clump.
15	,,	Early metaphase	,, ,,
63	4×10^{-6} M	Prophase. Nucleoli still present 15 min. later	Chromosomes clump. Cell oedema.
64	,,	Late prophase	Chromosomes clump. Small vacuoles in cell.
11	7×10^{-6} M	Metaphase plate	Complete mitosis but cells reunite.
10	,,	,, ,,	Chromsomes clump. Oedema.
12	,,	Early metaphase	Ditto.
13	,,	Late prophase	,,
14	,,	Early metaphase	,,

TABLE II.—*Dimethyl (XXVIII).*

Ref. No. of cell.	Concentration.	Stage applied.	Effect.
46	2×10^{-6} M	Prophase. Nucleoli still present 10 min. later	Chromosomes clumped.
47	,,	Ditto, ditto, 3 min. later	,, ,, and dispersed
45	,,	Late metaphase	Complete mitosis.
26	4×10^{-6} M	,, ,,	,, ,,
25	,,	,, ,,	,, ,, cell oedema.
24	,,	Early metaphase	Chromosomes clumped and dispersed.
21	8×10^{-6} M	Metaphase plate	Ditto.
22	,,	,,	Ditto. Cell oedema.
43	,,	Early metaphase	,, ,, ,,
44	,,	,, ,,	No reconstruction or cleavage after anaphase.

TABLE III.—*Methyl-free (II).*

Ref. No. of cell.	Concentration.	Stage applied.	Effect.
27	3×10^{-9} M	Late prophase	Completes mitosis. Partial secondary fusion.
26a	,,	Metaphase plate	Ditto
28	6×10^{-9} M	,, ,,	,,
30	1×10^{-8} M	Late prophase	,,
31	,,	Early metaphase	Complete mitosis. Full secondary fusion.
32	1×10^{-7} M	,, ,,	Completes mitosis. Partial secondary fusion.
33	,,	,, ,,	Ditto. Distorted cleavage.
34	5×10^{-7} M	Metaphase plate	,, Partial secondary fusion.
35	,,	,, ,,	,,
37	1×10^{-6} M	Late prophase	Arrest in metaphase for 42 min.
36	—	Metaphase plate	Completes mitosis. Partial secondary fusion; some oedema.
42	5×10^{-6} M	Early metaphase	Completes mitosis. Cleavage with blebs.
41	,,	,, ,,	Cleavage inhibited.
38	1×10^{-5} M	Late prophase	Death in metaphase. Oedema.

The clumping of chromosomes is an unspecific reaction evoked by many substances. There is evidence that disturbance of their normal water content is involved, for hypertonic saline can provoke this effect (Hughes, 1952*c*). The association of clumping with cell oedema in the action of Synkavit further suggests this possibility.

Inhibition or arrest in stages of mitosis other than metaphase has been seen in the course of the present work. Instances of arrest in early prophase have been observed with Synkavit (Fig. 10, 11) (49, 53). These cells remained in early prophase for periods of 20 minutes with nucleoli and nuclear membrane still present. Although it is not always easy to distinguish nuclei in early prophase from some intermitotic nuclei with conspicuous chromocentres, yet the distinction in these instances was made certain because in the film records the arrested nuclei in prophase revealed movement of their contents, which is often seen in this period of mitosis but not, however, in inter-phase. Arrest in early prophase of chick cells in culture is a rare phenomenon (Hughes, 1952*a*).

Inhibition of stages of mitosis subsequent to anaphase has been observed. In one instance (44) with the di-methyl derivative it was followed neither by nuclear reconstruction of the daughter chromosomes nor by cleavage of the cell (Fig. 6–9). Suppression of the latter event alone is much more common and leads to the production of a binucleate cell (Fig. 20). With high dilutions of the methyl-free compound, however, such cells may originate in another way; mitosis follows an apparently normal course, but after the cell has divided the daughter cells undergo a secondary fusion. Sometimes their reunion is only partial, but one

clear instance of complete fusion was recorded (31) (Fig. 27–31). The methyl-free substance exerted this effect in late telophase over a range of dilutions of about one thousand-fold (27, 3×10^{-9} M; 36, 1×10^{-6} M). At higher concentrations the substance rapidly kills both dividing and resting cells (38, 1×10^{-5} M).

It is noteworthy that although the effect of the methyl-free compound differs considerably from those of the methylated derivatives, both in the types of inhibition and the dilutions at which they are exerted, yet in the present data there are no significant differences in the maximum concentrations of each of the three substances at which a cell has been observed to undergo a normal mitosis (Synkavit, 17, 18, 19; di-methyl compound 25, 26; non-methyl 36, 42).

One point in the mitotic cycle on which these three substances do not appear to exert any immediate effect is the entry of cells into prophase. Early prophases are still found in cultures treated with these substances at the highest concentrations which have been used in the course of this work.

Since Synkavit and the di-methyl compound can interfere with the division of a cell in several ways, a culture which has been treated with these substances at appropriate dilutions for periods of an hour will show a variety of effects. These depend both on the individual sensitivity of the cells and the phase of mitosis which they had reached at the time of adding the substance. The fate of a particular cell selected for photography is, therefore, to some extent a matter of chance. It may be that the 55 instances which are recorded in Table I do not exhaust these possibilities, though study of the whole cultures after fixation has not so far revealed any other types of inhibition.

The variability of the behaviour of cells towards these substances, however, extends further than to inhibition at different stages of mitosis. Treatment of a culture with a concentration sufficient to arrest and damage some dividing cells will leave unaffected others which continue a normal mitosis. This fact can best be illustrated by comparison of the effects of Synkavit in a number of instances where it was applied to cells early in prophase. Thus at 5×10^{-7}M there occurred both a normal mitosis (56) and an arrest in early prophase (53). At 1×10^{-6} M a normal mitosis (59), an arrest in metaphase (60) and a clumping of chromosomes and cell oedema at this stage were recorded (52). When the di-methyl compound at 4×10^{-6} M was added to cells in metaphase, there were seen both a normal mitosis (26) and an instance of clumping of the chromosomes (24). This circumstance must be borne in mind in the search for any further generalisations from these data. However, one other conclusion clearly emerges, namely, that the effect of these substances on dividing cells is greater when they are applied at early stages of mitosis. An illustration of this fact can be given with each of the three compounds. With Synkavit at $3{\cdot}5 \times 10^{-6}$ M addition to a cell early in metaphase resulted in clumping of chromosomes and oedema (15), while later in metaphase in two instances this concentration was not found to be inhibitory (18, 19). With the di-methyl compound (XXVIII) at 2×10^{-6} M the same distinction was found between cells treated early in prophase (46 and 47) and another to which the compound was added during metaphase (45). Again, the methyl-free derivative (II) showed the same difference at 1×10^{-6} M (37 compared with 36).

These observations point clearly to the conclusion that a period of time which is an appreciable fraction of the duration of mitosis is required for these substances to exert their effect on the dividing cell.

DISCUSSION.

In earlier studies of the effects of inhibitory substances in which the same technique and biological material were used certain regularities were usually observed (Hughes, 1950, 1952*a*). They can be summarised in this way.

1. In most instances one or more phases of mitosis are usually specially sensitive to the effects of an inhibitor. Cells may be prevented from entry into mitosis; often the development of the spindle is readily suppressed; cleavage and nuclear reconstruction may sometimes be affected. Usually, however, once a cell has entered prophase it is relatively insensitive to an inhibitor until this phase of mitosis is passed.

2. It is probably true to say that phase specificity is most marked with substances to which the cell in division is much more sensitive than during the resting stage. Thus, for instance, the action of colchicine on the dividing cell is restricted to its effect on the mitotic spindle, and this is exerted at dilutions much greater than those necessary to affect intermitotic cells. On the other hand, substances such as urethane, fluoride and cyanide affect several phases of mitosis rather unspecifically, and only at dilutions near those which are toxic to intermitotic cells. Such substances have but little claim to the title of "mitotic poisons".

Although Synkavit and the compounds related to it affect dividing cells at considerable dilutions, yet these are nevertheless similar to the concentrations which rapidly provoke oedema in intermitotic cells. Moreover, they show unusually little phase specificity in their action on mitosis. Although the effect most frequently seen is the inhibition of the mitotic spindle and clumping of the chromosomes in metaphase, yet instances have been observed in the course of the present work of mitotic inhibition during prophase, and also in anaphase and telophase. These substances, however, seem without any marked immediate effect on the entry of cells into mitosis.

It is of interest to compare the results obtained in the present work with those obtained by Mitchell and Simon-Reuss (1947, 1952*a*, 1952*b*), in which the same compounds were applied to tissue cultures of chick fibroblasts for much longer periods. In their work the substance was mixed with the medium of the culture at the time of explantation. The culture was fixed after 24 hours, and in the stained preparations counts were made of the total number of dividing cells in each phase of mitosis, both normal and abnormal.

With Synkavit the concentrations which both provoke cell oedema and affect mitosis within the first hour do not appear to differ greatly from those with which these effects are seen after 24 hours of treatment. In the present work the least concentration at which chromosomes were clumped was 1×10^{-6} M; at $3{\cdot}5$–$4{\cdot}0 \times 10^{-6}$ M this effect was produced several times, whereas after 24 hours' exposure to this concentration 15 per cent of dividing cells in stained preparations were abnormal. Again, the present results suggest that no single phase of mitosis is specially sensitive to the influence of Synkavit; furthermore, neither is there any disturbance of the normal phase distribution after treatment for 24 hours.

With the di-methyl derivative (XXVIII) the same correspondence holds good in the effective concentration in the two sets of experiments; the abnormal mitotic figures are seen in both when the substance is applied at 2×10^{-6} M.

Later, however, it causes a cumulative arrest in metaphase, for in the stained preparations 60 per cent of the dividing cells present were in this stage of mitosis after 24 hours.

The methyl-free compound II shows certain further differences in behaviour. The present observations have shown that within the first hour no effect on the course of mitosis earlier than late telophase is observed with concentrations of 1×10^{-7} M and below. However, after treatment for 24 hours with even greater dilutions a very marked accumulation of cells in metaphase was found, together with oedema of the intermitotic cells. The proportion of cells entering prophase has then decreased. Thus, this substance appears to penetrate the cell membrane so slowly that a concentration within the cell sufficient to provoke these effects is not reached for several hours. The secondary fusion of the daughter cells in late telophase which has been observed in the course of the present experiments may well be due to an alteration in the properties of the cell surface. A large number of binucleate cells were seen in stained cultures after treatment for 6 hours with this substance at concentrations of 1×10^{-9} M. This effect may be due either to secondary fusion of the daughter cells, or to an inhibition of cell cleavage in the first instance.

Finally, the value of the two general methods of investigations of the action of chemical substances on living cells in culture can be compared. In the one mainly used in the present work the effects of the agent on a single living cell in the culture is followed; in the other a quantitative analysis of the whole culture is made after fixation and staining. We would suggest that both methods are complementary and that neither is a substitute for the other. Where the effects of a given treatment often differ from cell to cell, it would be unwise to draw conclusions from the study of only a few examples. Here the quantitative analysis of large numbers of cells is indispensable. On the other hand, the close study of the course of events in the living cell offers some hope for the further understanding of the mechanism of action of an inhibitory substance. This task is, of course, far more difficult than the tracing of the visible course of events and here very much yet remains to be done.

SUMMARY.

1. The immediate effects of three phosphorylated naphthohydroquinones have been studied on living cells in mitosis in cultures of chick osteoblasts. These compounds are Synkavit (2-methyl-1:4-naphthohydroquinone diphosphate), and the related di-methyl and methyl-free derivatives.

2. They have been applied at dilutions ranging from 3×10^{-9} M to 1×10^{-5} M for periods up to one hour. The immediate effects of such treatment have been recorded by phase-contrast cinémicrography.

3. Cells may be inhibited at any point in mitosis, though their entry into prophase is apparently unaffected under the conditions of these experiments. Arrest in metaphase and clumping of the chromosomes is the most common effect. Individual cells vary greatly in their sensitivity to these substances. The severity of the effects of a given dilution is proportional to the time for which it is applied.

4. The maximum concentration of each substance which has allowed a cell observed in a treated culture to undergo a normal mitosis is of the same order for all three compounds.

5. The reactions of dividing cells to the two methylated compounds are more clearly allied than those evoked by the methyl-free derivative. At sub-lethal concentrations the latter does not affect the course of mitosis until late in telophase, when to a varying extent the daughter cell re-unites.

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A COMPARISON OF DIFFERENT PROTEIN FRACTIONS OBTAINED FROM THYMUS NUCLEI ISOLATED IN AN AQUEOUS MEDIUM.

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RESULTS for the analysis of thymus histone presented from this laboratory (Hamer, 1950, 1951) have been followed by similar results from other sources (Daly, Mirsky and Ris, 1951 ; Eadie and Leaf, 1952). These results all show the same general characteristics in composition but the actual values obtained vary appreciably from worker to worker, suggesting that either the methods of isolation or the source of the material may be affecting the final product. Analysis of other nucleo-proteins have been reported by Brunish, Fairley and Luck (1951), liver histone, and by Khouvine and Baron (1951), rat epithelioma histone. In the last-mentioned paper evidence was presented that different treatments during isolation could yield products of varying composition. Stedman and Stedman (1951) have also reported qualitative and partial quantitative analyses of a number of basic proteins from isolated nuclei.

In the work reported here further analyses of thymus histone are presented along with other results for the non-histone or acid-insoluble protein of the nucleus. All the fractions examined were derived from the same batch of thymus nuclei, and consequently it was possible to study whether the methods of fractionation used caused changes in the amino-acid compositions. A preliminary account of this work has already been presented (Hamer, 1952).

EXPERIMENTAL.

Isolation of nuclei.

The nuclei were isolated from about 1½lb. of defatted calf thymus by the following method : The tissue was homogenised in a Blendor, suspended in a total of 2 litres of saline citric acid solution (0·14 M NaCl, 0·025 M citric acid), and then centrifuged, discarding the supernatant. The sedimented material was washed by alternate suspension and centrifugation in another saline citric acid solution (0·14 M NaCl, 0·01 M citric acid), six times in all, The preparation of isolated nuclei was then free of whole cells and had no appreciable cytoplasmic contamination. The nitrogen to phosphorus ratio of such a preparation was 4·5 : 1. All these operations and those described below were carried out in the cold below 5°.

Preparation of the different fractions.

(*a*) Whole nuclear protein : A part of the above preparation of nuclei was suspended in 0·8 M sodium chloride and brought to about pH 6·5. After standing

overnight the suspension was centrifuged for 5 minutes at 2000 r.p.m. to remove any large undissolved portions. Next a tenth of the volume of a 5 per cent solution of sodium dodecyl sulphate (sulphonated lauryl alcohol-Glover) in 45 per cent alcohol was added and the pH brought to 5·5. The mixture was stirred for 3 to 4 hours and then allowed to stand for 48 hours, when the flocculent precipitate which had formed was centrifuged off and washed twice with 0·8 M saline containing a twentieth of a volume of the sodium dodecyl sulphate solution. Finally it was suspended in distilled water, dialysed and freeze-dried (Fraction DP). The supernatant from the precipitation contained nucleic acid, which was obtained in fibrous form by adding alcohol.

(*b*) Histone and non-histone fractions: The remaining part of the preparation of isolated nuclei was suspended in 1·2 M sodium chloride containing M/20 phosphate buffer at pH 6·5. This suspension was left for 24 hours with occasional mechanical stirring and the opalescent solution obtained was centrifuged. The residue was washed several times, combining the supernatants, until there was left only a small amount of insoluble material (Fraction R).

Half of the nuclear solution thus obtained was worked up by the method described previously (Hamer, 1951), namely precipitation of the nucleoprotein by dilution followed by resolution and reprecipitation and then extraction of the nucleoprotein with 0·1 M hydrochloric acid. The acid extract was dialysed and the histone fraction obtained by the addition of ammonia. The histone was redissolved in acid, dialysed against tap water and distilled water, and finally freeze dried (Fraction NH). The residue from the acid extraction was washed two further times with 0·2 M hydrochloric acid, and the remaining insoluble fibrous material was suspended in water, dialysed for 48 hours and freeze-dried (Fraction NR).

The remaining half of the solution of nuclei in 1·2 M saline was shaken with an equal volume of chloroform-butyl alcohol (4 : 1) to dissociate the nucleic acid and the protein. The nucleic acid remains in the aqueous phase (from which it may be precipitated with alcohol), while the protein forms a gel at the interface. This treatment was repeated twice, combining the gel layers obtained. The gel was washed three times with the buffered 1·2 M saline, agitating and centrifuging each time, and then the protein was thrown down by adding chilled alcohol. The precipitate was extracted overnight with 0·1 M hydrochloric acid, and the acid extract containing histone was worked up as above to give Fraction SH, the second histone specimen. The residue was washed further with acid and then suspended in water, dialysed and freeze-dried (Fraction SR).

These four main fractions and the " whole protein " (DP) were then analysed. There was insufficient material in Fraction R to permit a full analysis, but it is hoped to study this further at a later stage. The scheme of fractionation is given in Fig. 1, and shows how the different fractions are derived from the one preparation of nuclei.

Analytical methods.

General amino-acid analyses were carried out by the method of Moore and Stein (1948, 1949) after hydrolysis with redistilled 6 N hydrochloric acid for 18 hours under reflux. The details of the method used were given in earlier work. The separation of the leucine fraction was not carried out, and so the results for

leucine and isoleucine are reported together. In certain cases the chromatographic results were inadequate and it was necessary to do independent chemical

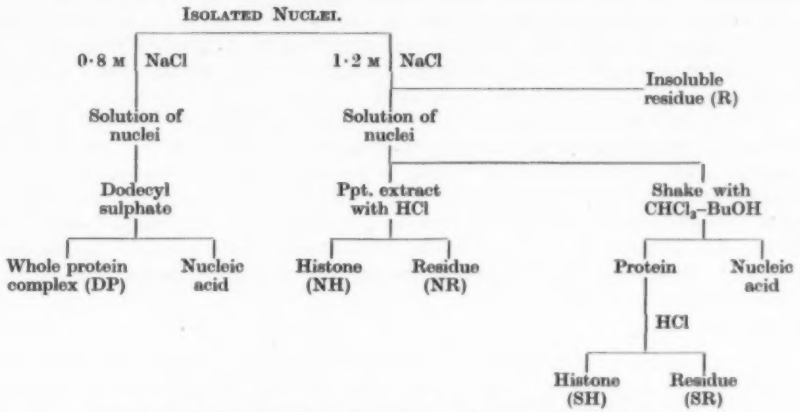

Fig. 1.—Method of fractionation of the isolated nuclei by which the different proteins analysed were obtained.

estimations. Methionine was not distinguished chromatographically, but small amounts could be detected and estimated in certain fractions by the method of Horn, Jones and Blum (1946), using the reaction with nitroprusside and phosphoric acid. Tryptophane was estimated without prior hydrolysis of the protein using p-dimethylaminobenzaldehyde in sulphuric acid followed by nitrite (Spies and Chambers, 1948). Various tests for cystine were tried, including the polarographic method (Stern, Beach and Macey, 1939), the Fleming reaction using dimethyl-p-phenylenediamine (Block and Bolling, 1951, p. 205) and the Folin-Winterstein reaction (Shinohara modification, Block and Bolling, 1951, p. 194). The last named was found the most satisfactory for these fractions as the other methods gave only approximate results. Breakdown products of the nucleic acid in some fractions interfered with the polarographic method, while in the Fleming reaction the optimum reaction times varied for different specimens.

Nitrogen was estimated by a micro-Kjeldahl method and phosphorus by Holman's (1943) method after perchloric acid combustion. The recoveries of protein nitrogen as amino-acid nitrogen were of the order 97 to 98 per cent.

RESULTS.

General properties of the fractions.

The two histone fractions were quite typical in their properties. They were light and white when dry and readily dissolved in water or acid, being precipitated out by adding ammonia to pH 9-10. The specimens had slightly lower nitrogen contents than those previously examined and contained about 0·2 per cent phosphorus.

The specimen SR of non-histone or acid-insoluble protein was slightly yellowish, and contained a little less nitrogen and more phosphorus than the histones. It was insoluble in water, but would dissolve at pH 9-10 to give a pale yellow solution

and then precipitated out when this solution was acidified to about pH 5·5. In the course of this treatment some unit in the protein is apparently destroyed as evidenced by the production of a strong unpleasant odour on acidification. The related specimen NR contains, of course, much nucleic acid in addition to protein. Estimated from the phosphorus content and the absorption spectrum there is approximately 56 per cent nucleic acid in this fraction. In dilute alkalis an opalescent viscous solution is obtained.

Finally, specimen DP precipitated from the solution of isolated nuclei by sodium dodecyl sulphate has a nitrogen content of about 9·8 per cent indicating that the ratio of protein to detergent is around 3 : 2 by weight. The linkage between the detergent molecules and the protein seems very firm and so far it has not been possible to split the two. Extraction with acid will not remove any histone, while in electrophoresis on paper at pH 8·7 the complex moves mostly as a single spot with about the same mobility as serum albumin.

Analytical results.

The full amino-acid analyses are recorded in Table I along with the nitrogen and phosphorus contents. The results for Fraction NR have been calculated assuming full recovery of the protein nitrogen to bring the results into line with the other fractions.

TABLE I.—*Amino-acid Analyses.*

Fraction.	NH.	SH.	NR.	SR.	DP.
Alanine	6·4	6·6	6·0	5·2	5·6
Ammonia	4·0	2·6	7·8	3·2	4·1
Arginine	28·9	30·9	20·0	23·5	25·4
Aspartic acid	3·6	3·0	5·7	5·1	4·2
Glutamic acid	6·4	6·1	7·2	8·8	6·6
Glycine	6·1	5·8	8·4	6·1	5·8
Histidine	4·0	+	4·0	4·2	4·6
Leucine	9·7	10·4	9·7	10·0	9·8
Lysine	10·4	11·7	7·5	8·5	10·2
Phenylalanine	2·0	1·1	2·3	2·1	2·1
Proline	2·5	3·0	3·7	3·2	2·7
Serine	2·8	3·2	4·0	3·9	3·5
Threonine	3·9	4·0	4·2	4·3	4·0
Tyrosine	1·8	1·4	2·3	2·1	2·3
Valine	5·2	4·2	5·3	5·5	5·3
*Cystine	0·02	−	+	0·24	+
*Methionine	0·63	×	×	1·1	+
*Tryptophane	0·14	×	0·65	1·0	+
% Nitrogen	17·1	17·0	13·9	15·6	9·8
% Phosphorus	0·20	×	5·2	0·78	0·48

Amino-acid nitrogen expressed as percentage of protein nitrogen. ×, Not estimated; −, absent; +, present, but not determined quantitatively.

* Estimated by colorimetric methods.

The compositions of the two histone fractions are in fair agreement, and there is certainly nothing to suggest that the two methods of preparation used here give different products. There are, however, some variations from the results given in the earlier work, notably the values for leucine and glutamic acid. About 1 per cent of methionine was found to be present by separate colorimetric analysis, although it was not detected by either qualitative or quantitative chromatography.

Cystine or tryptophane were either absent or present only in extremely small amounts.

Of the two non-histone proteins, characterised by their acid insolubility, the most striking feature is the close similarity in amino-acid composition. When it is remembered that the histones have an isoelectric point about 9·5 and the non-histone fractions an apparent isoelectric point of 5·5 this similarity is surprising. One definite and probably important difference is that these non-histone proteins contain small but appreciable amounts of cystine and tryptophane. It would seem then that the difference in the two groups is one of structure due to the presence of these amino-acids in the one type and not in the other. This will be considered further below. Both of the fractions contained less arginine and lysine but slightly more glutamic and aspartic acids than were found in the histones.

The results of the analysis of the Fraction DP which contains both the protein types analysed gave, as would be expected, a composition intermediate between the two.

DISCUSSION.

Considering first the two specimens of each group, there is no evidence that the two methods of preparation used here give different products providing the same starting material is used. It would therefore seem more likely that the differences between the results of various workers for the analysis of histone specimens are due to variations in the original animal material. However, it must be borne in mind that Khouvine and Baron (1951) found that calcium chloride precipitation yielded products of varying composition, while Stedman and Stedman (1950,1951) have put forward evidence for the presence of subsidiary histone fractions.

It has already been pointed out that the most important difference between the histone and non-histone proteins of the nucleus seems to be the presence of cystine and tryptophane in the latter. The act of separating the non-histone protein from the nucleic acid apparently damages the protein, and it does not seem possible to obtain it in other than an insoluble and denatured form. This would suggest that the non-histone protein may be chemically bound to the nucleic acid while the histone is held by a predominantly ionic union. Some support for such a structure has recently been presented by Fleming and Jordan (1952), who have found that the results of electrophoretic studies on thymus nucleoprotein in solutions of varying ionic strength could be explained by the existence of an equilibrium $NP \rightleftharpoons Np + p'$ (where NP is the original nucleoprotein, Np a nucleoprotein fraction with a higher nucleic acid content, and p′ a dissociated protein). However another possibility is that in the cell the histone is loosely combined with the non-histone protein and that acid treatment breaks the compound open. This would be in accord with the close similarity of amino-acid composition, but would require that all the cystine and tryptophane remain with one fraction on fission. The first possibility considered seems on the whole to be more probable, as it is more in keeping with the variation in the amounts of histone and non-histone protein in the nucleus of different cell types (Hamer, 1951 ; Stedman and Stedman, 1951 ; Mirsky and Ris, 1951). In the case of the thymus nuclei studied here the histone and non-histone proteins occurred in roughly

comparable amounts as judged by the changes in phosphorus content during fractionation. This is in agreement with results of Stedman and Stedman (1951).

Possibly then all or part of the protein that has been referred to in this paper as non-histone protein is combined with nucleic acid to form the structure of the chromosome material. Whether the histone is really an essential part of the chromosome remains to be proved, for, as Stedman and Stedman (1951) have pointed out, it does not seem reasonable to presume that the chromosomes occupy the whole nucleus. It would also be unjustifiable to assume that all the proteins were chromosome constituents. In this respect it will be important in the future to obtain further information on whether (*a*) appreciable amounts of protein are lost during the isolation of nuclei in aqueous media as has been reported (e.g., Kirkham, 1952), and (*b*) the residual insoluble Fraction R in the above experiments is derived from the nuclei or is extraneous material such as fibrous tissue.

SUMMARY.

1. The preparation of histone and non-histone protein fractions from isolated nuclei of calf thymus gland is described.

2. Complete amino-acid analyses of these fractions are reported and the compositions of the histone and non-histone types are compared.

3. The possible organisation of these proteins along with nucleic acid in the nucleus and chromosomes is briefly discussed.

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SURVEY OF PAPERS.

CLEMMESEN, NIELSEN and JENSEN find that the increased mortality from cancer of the lung among men in Denmark is due to a real increase in incidence of the disease. They suggest that this is due to some carcinogenic influence, other than atmospheric pollution, which began to exert its effect in Copenhagen between 1900 and 1910, and affected the provincial towns and rural areas some years later (p. 1).

KENNAWAY and KENNAWAY find that the incidence of cancer of the lung in coal miners is less than in the general population, and discuss some possible reasons for this. They also find differences between the incidence of lung cancer in face-workers and other miners, and between workers in the various coalfields (p. 10).

HARNETT has studied the 5-year survival rates of 2880 cancer patients in relation to the delay in commencing their treatment. He divides the sites of the disease into three groups: A, those in which the highest percentage of 5-year survivals are among the patients treated within the first 6 months; B, those in which it is among those treated 6–12 months from the first sign, and C, in which the highest percentage of 5-year survivals are among those treated after a delay of a year or more (p. 19).

KREYBERG and CHRISTANSEN find the prognosis in a series of small carcinomas of the breast to be no better than the average (p. 37).

SHAPIRO, KEEN, COHEN and MURRAY give an account of a series of skin tumours occurring in Bantus and contrast the incidence of the various types with that found in other races (p. 45).

FOURACRES and WHITTICK draw attention to the close similarity in structure and natural history between molluscum sebaceum and the lesions described as multiple spontaneously healing epithelioma, and suggest that the two conditions are identical (p. 58).

CHRISTIE describes a case of multiple primary cancer in an elderly male, the tumours being in the floor of the mouth and in the duodenum (p. 65).

NORDLING puts forward the theory that the cancerous cell contains not one but a number of mutated genes. The occurrence of such accumulations of mutations may be expected to increase according to a certain exponent of age, as well as according to the increase of cell proliferation (p. 68).

DMOCHOWSKI gives an account of the mammary tumours resulting from forced mating of the hybrid females resulting from crossing agent-free females with agent-harbouring high-cancer strain males. He also gives the results of biological tests for the presence of the mammary tumour agents in some of these tumours, as well as a study of the microscopic appearance of the breast cancers in these mice (p. 73).

PEACOCK and PEACOCK describe three new chemically induced fowl sarcomata which, though readily transplantable, could not be transmitted by cell-free preparations (p. 120).

CARR gives an account of the behaviour of transplants of the chemically-induced GRCH/15 fowl sarcoma. He emphasises and discusses the difference between this transplanted fowl tumour and comparable growths in rodents (p. 131).

WILTSHIRE has analysed one sample of normal tissue and four samples of tumour tissue for isomers of glutamic acid. He finds the percentage of D-glutamic acid is very small in

all cases, and not more than would be formed by inversion of the L-isomer during hydrolysis (p. 137).

HUGHES and SIMON-REUSS give an account of the effects of three pH phosphorylated naphthohydroquinones on living cells in mitosis in cultures. Arrest in metaphase and clumping of the chromosomes is found to be the commonest effect, though individual cells vary greatly in their sensitivity (p. 142).

HAMER describes the preparation of histone and non-histone protein fractions from isolated nuclei of calf thymus glands and reports complete amino-acid analyses of these fractions (p. 151).